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VOLUME 12 APRIL, 1935 NUMBER 4

THE LARVAL DEVELOPMENT OF BRITISH COLUMBIA BRACHYURA

I. XANTHIDAE, PINNOTHERIDAE (IN PART) AND GRAPSIDAE[1]

BY JOSEPHINE F. L. HART[2]

Abstract

The larval stages of four species of crabs inhabiting British Columbia are described from series obtained by hatching eggs in the laboratory. In the development of *Lophopanopeus bellus* (Stimpson) there are found to be four zoeal stages, of *Pinnotheres taylori* Rathbun two, and of *Hemigrapsus nudus* (Dana) and *H. oregonensis* (Dana) five. There is only one megalopal stage in each of the species.

Knowledge of the life history of Pacific coast decapod Crustacea is very limited. The free-swimming larvae form an important part of shallow water plankton, and as such are a component of the food of fish and macro-plankton. Although the larvae occur in great numbers near the shore, comparatively little is known of their development, owing perhaps to the fact that it is usually necessary to rear the stages to be at all sure of correct identification, as the larvae are so unlike the adults and in many cases so like one another. They are difficult to rear in artificial conditions, and as far as the writer has been able to determine, complete series of only four species have been recorded to date. In this paper all the larval stages of four more species that have been reared from the egg are described. Various authors have described series, taking the stages from plankton, but this method is obviously less accurate than rearing them from the egg.

Lebour's paper (10) is invaluable for the study of early Brachyuran development. It is a brief monograph of the larval stages of some 35 species of crabs inhabiting the Plymouth area. In America, Hyman (5-7) gives summaries of work done previously by others, and makes original contributions to the knowledge of the larvae of the families Xanthidae, Pinnotheridae and Grapsidae. Aikawa (1, 2) describes some of the zoeal stages of a large number of Japanese crabs, dealing particularly with the determination of characters

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that might be used for classification. There are a number of other papers describing forms found on both sides of the Atlantic ocean, but the works of these three authors have been of most value in the study of crabs found in British Columbia.

Methods of Rearing

The chief difficulty encountered in rearing decapod larvae is the maintenance of a constant supply of suitable living food. Many of the forms eaten in the natural state, when placed under laboratory conditions, soon die and their decomposition products in the water kill the larvae. The life histories of so few forms in this locality have been worked out that there are practically no records of the spawning periods of the invertebrates, and therefore it is difficult, without considerable research, to determine what larvae are available at the different seasons. Lebour (8-10) was successful in rearing three species of crabs in "plunger-jars" on a diet of *Ostrea*, *Teredo*, *Echinus* and *Pomatoceros*. The megalopa and young crabs were fed on small pieces of the mantle of *Mytilus edulis*. The writer has found the larvae of the native oyster, *Ostrea lurida* Carpenter, satisfactory for feeding, as the larvae are retained for some time in the mantle cavity of the adult and therefore can be obtained in quantity, yet are free-swimming when placed in the water. The veliger larvae of Nudibranchs and the trochophore larvae of the Japanese oyster were also used, but did not prove as suitable as those of the native oyster. The megalopa and young crabs ate finely minced muscle of various molluscs.

Berried females were collected and placed in aquarium jars. When the eggs hatch, the larvae swim to the surface of the water and may be removed with a pipette and placed in large beakers of sea water containing food. The water was aerated by stirring with a glass rod. The larvae were examined daily and fresh water and food given if required, and all dead material and sloughed skins removed. Records of pigmentation were kept and specimens of each stage, as well as cast off skins, preserved.

The Developmental Stages

Few records have been obtained of the length of time necessary for the embryonic development of decapods. According to the writer's observations seven weeks is the shortest period during which the eggs were carried attached to the pleopods of the female, and thus protected, kept free from encrustations and debris, and aerated. The length of time seems to be proportional to the size of the adult. The size of the egg mass and the average size of the eggs vary greatly with the different species.

When the egg hatches, the last embryonic stage, the prezoea or protozoea, is set free. This stage may last for some hours, or in exceptional cases, days, but usually is replaced by the first larval stage soon after hatching. The prezoea is enclosed in a thin membraneous cuticle covering the entire body and appendages. In many species this membrane is produced, and delicately plumed, beyond the invaginated tips of the antennules, antennae and telson.

The spines of the carapace, which are present in the first zoea, are held flat against the body by the cuticle in the prezoea and the setae and spines are invaginated upon themselves. The prezoea is able to swim by jerky movements of the abdomen, similar to the movements of gnat pupae. In all the Grapsoid crabs that have been observed to hatch from the egg, the prezoea lacked the prolongations of the membrane found in other forms.

When the embryonic cuticle is cast off, the first zoea swims to the surface of the water by the powerful beating of the exopodites of the first two pairs of maxillipedes. The zoea has a well developed carapace, which may be produced into a rostral, a dorsal and paired lateral spines. There usually is a pair of short setae on the dorsal part of the carapace, above the heart. The abdomen of the first stage is composed of five segments and the telson. The sixth segment may become separate from the telson in a later stage. The thoracic appendages consist of a pair of large compound eyes, tubular antennules, antennae, bilobed mandibles, two pairs of lamellar maxillae and the first two pairs of maxillipedes. The exopodites of the maxillipedes are equipped in the first zoea with four two-jointed, long, plumose setae. The remainder of the thoracic appendages appear as small knobs posterior to the maxillipedes and are covered by the carapace. There are no functioning appendages on the abdomen, but paired swellings may be present on the second to the fifth segments—the pleopods of the post-larva. There is always a lateral knob on either side of the second abdominal segment, and there may be similar, usually smaller ones, on the third, fourth or fifth segments. There may be spines on the lateral posterior angles of the second to fifth segments. The telson is typically bicornuate, with three pairs of internal setae and varying numbers of external spines. The number of internal setae often increases in the later stages and the spines may decrease in number.

When the first zoea moults and the second emerges, the number of setae present on the appendages is found to have increased in most cases. Six is the usual number of setae on the exopodites of the swimming maxillipedes in the second zoea, eight in the third, ten in the fourth and twelve in the fifth. In those forms having only two zoeal stages the non-functioning appendages are of the same degree of development as those of the last zoea of the forms with four or five stages.

The last zoea metamorphoses into the post-larva; the megalopa. It resembles the adult crab in shape and the appendages are all functional. The megalopa can swim freely through the water by means of well developed pleopods, bearing long plumose setae. The abdomen may be flexed under the thorax when the pleopods are not in use. There may be large spines on the carapace and hook-like knobs on the coxae of some of the periopods. In all those crabs that have been reared from the egg only one megalopal stage has been found, the first young crab stage appearing after the first moult of the megalopa. The first young crab stage is like the adult in structure, but usually of different shape and proportions. By subsequent moults the specific characters of the adult are gradually assumed.

Decapod larvae are usually very transparent when alive. The amount and color of the pigment varies according to the degree of contraction of the chromatophores. In some forms the chitin itself may be tinged with bright colors. As the color does not preserve well, it is only of value in preliminary sorting of living or recently killed material, especially in those forms that are morphologically similar but differ in coloration.

The length of time spent in each stage seems to depend considerably on the relative abundance of food, the temperature and salinity of the water and other such external conditions. The first zoeal stage, in suitable natural conditions probably lasts for two or three days, and the time spent in each stage increases as the larva grows. Under laboratory conditions, four to five weeks is usually required for development from the egg to the young crab stage.

The larvae vary considerably in size, so that measurements are only relative. That this variation is not entirely due to the food supply is shown by the lack of uniformity in size and color of the brood hatched by one female.

In attempting to find characteristics suitable for classification, it is necessary to use those that do not become greatly modified as the animal develops. The spines of the carapace, the armature of the abdomen and telson, and the form of the antennae are perhaps the most important. Aikawa (2) uses the number of setae on the endopodites of both maxillae and of the second pair of maxillipedes. The form of the rostrum, the spines on the carapace, the presence of sensory setae on the last pair of legs, and the number of setae on the exopodites of the uropods are characteristic of the megalopae.

Family Xanthidae

The larval development of a number of species of the family Xanthidae has been followed by other workers. Hyman (7) describes some of the stages of forms found at Beaufort, North Carolina, and compares them with European species described by other authors. Lebour (10) gives the characters of the larvae of the family and the description of stages of three species. Connolly (4) gives an account of the larval stages of *Rhithropanopeus harrisi* (Gould) from New Brunswick. On the Pacific, Aikawa (1) studied the first zoeae of two species of *Xantho* found in Japan.

The zoeae and megalopa of *Lophopanopeus bellus* (Stimpson) described below, fit into Lebour's (10) characterization of the larvae of the family Xanthidae, sub-family Xanthinae, with the exception of the number of lateral spines on the telson of the zoeae. The telson resembles that of *Rhithropanopeus harrisi* (Gould), in having only one lateral spine on each fork.

Lophopanopeus bellus (Stimpson)

Eggs carried in April; hatching May to August, changing in color from deep purple to light brown, and increasing in size from 0.33 to 0.41 mm. in diameter.

Typical prezoea, four zoeae and one megalopa. First young crab stage obtained in laboratory five weeks after eggs hatched. Second died five weeks later when attempting to cast its skin.

Prezoea similar in color to first zoea. Spines of carapace flattened against body. Two embryonic spines, of unequal length, enclose antennule. Exopodite of antenna covered by four hairy projections of cuticle. Telson with seven embryonic spines on each side, all but fourth plumose.

First zoea (Fig. 1, *A*) 1.5 mm. (measured from tip of the telson fork to front of head) and 1.5 mm. from tip of dorsal spine to end of rostral. Body tinged with yellow, and rostral spine, antennae and telson forks with russet. Black chromatophores on bases of antennules, on mandibles and maxillae, at bases of lateral and dorsal spines, on postero-lateral part of carapace, on distal part of bases of first and second maxillipedes, and around proximal part of intestine. Pair of chromatophores present at junction of all abdominal segments. A red pigment spot on dorsal spine, a pair on first abdominal segment and on telson.

Dorsal and rostral spines long and tapering, laterals short. No setae on margin of carapace. Abdomen (Fig. 1, *B*) composed of five segments and telson, with knobs on third as well as second segment, and sharp points on postero-lateral margins of third, fourth and fifth. Dorsal part of each fork of telson with one spine and the usual six setae on internal margin.

Two long aesthetes and one seta on tip of conical antennule (Fig. 1, *C*). Antenna (Fig. 1, *D*) characteristic of family: protopodite swollen and produced into a long tapering process, sub-equal to or slightly longer than rostral spine; exopodite minute, about 0.025 mm. long with a short hair at tip. Mandible (Fig. 1, *E*) bilobed; incisor cut into two teeth and molar with a broad cutting surface. Maxillule (Fig. 1, *F*) rather narrow; endites of protopodite with 8 and 4 bristles, segments of endopodite with 1 and 6. Maxilla (Fig. 1, *G*) has 7 and 9 setae respectively on coxopodite and basipodite, 8 on endopodite and 5 soft hairs on scaphognathite.

First maxillipede (Fig. 1, *H*) typical, with 8 bristles on basis, 4 natatory hairs on exopodite and 2, 2, 1, 2 and 5 on the five joints of endopodite. Second maxillipede (Fig. 1, *I*) with 4 bristles on basis, 4 on exopodite and 1, 1 and 5 on endopodite. The remaining thoracic appendages are small and there is no indication of pleopods on the abdomen.

Second to Fourth Zoeae

Second zoea (Fig. 1, *J*) about 2 mm. long and 2.1 between tips of spines; third zoea (Fig. 1, *K*) 2.8 and 3.1 mm., and fourth (Fig. 1, *L*) 3.0 and 3.5 mm. As the zoea develops the dark chromatophores become more distinct and branched. Color of dorsal spine and antennae becomes concentrated in a russet band almost at tip of spines. With live material these brightly colored bands on the transparent spines serve as a distinctive character, which is unfortunately lost on preservation.

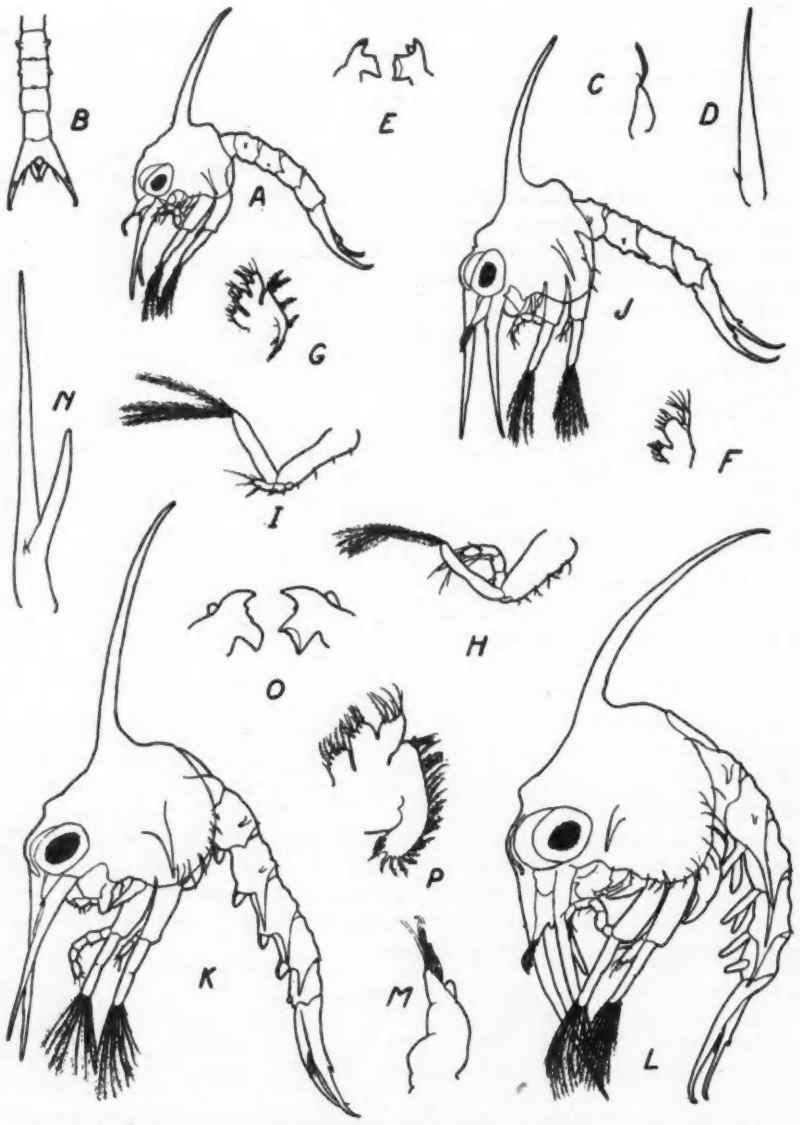

FIG. 1. *Lophopanopeus bellus* (Stimpson). *A, first zoea* × *30; B, abdomen* × *30; C, antennule* × *60; D, antenna* × *60; E, mandibles* × *60; F, maxillule* × *60; G, maxilla* × *60; H, first maxillipede* × *60; I, second maxillipede* × *60; J, second zoea* × *30; K, third zoea* × *30; L, fourth zoea* × *30; M, antennule* × *60; N, antenna* × *60; O, mandibles* × *60; P, maxilla* × *60.*

As the zoea grows the spines on the carapace and abdomen become much elongated. The base of rostral spine is produced laterally to form a shelf curved over bases of eyestalks and there is a distinct median ridge dorsal to this on the carapace. Rostral spine and processes of antennae are subequal in second zoea but in later stages rostral spine over-reaches antennae. Dorsal spine more than doubles in length during development but lateral spines become less conspicuous as zoea grows. Plumose hairs on margin of carapace increase from two in second zoea to about 12 in last. Sixth abdominal segment becomes separated from telson in third stage, although indication of the segmentation may be seen in second. Lateral marginal teeth on third, fourth and fifth abdominal segments gradually increase in size until they become longer than the segments against which they lie. An additional pair of small setae appear on internal margin of telson in third zoea.

Aesthetes of antennule increase in number during development; endopodite in fourth zoea (Fig. 1, *M*) can be seen as small knob, and basal part of appendage becomes inflated. The anlage of flagellum of antenna first seen in third stage; in last zoea (Fig. 1, *N*) it is less than half length of protopodite process. A small, unarmed palp present on mandible (Fig. 1, *O*) of last zoeal stage. Setae on inner margin of protopodite of maxillule increased in number, with a densely plumose seta appearing on outer margin in second stage and a bristle in addition in third; endopodite with 1 and 6 setae in all stages. Maxilla (Fig. 1, *P*) large and lamelliform, with increasing numbers of setae on all parts except endopodite, which bears 8 setae throughout. Swimming setae of maxillipedes increase to 6 and 7 in second zoea, 8 and 9 in third, and 9 and 11 in fourth. The slight division into two parts of third maxillipede and chela which can be seen in second zoea, becomes quite distinct in third. All thoracic appendages well developed in fourth zoea although not functional.

Megalopa. (Fig. 2, *A*)

Length about 2.8 mm., carapace 1.5 by 1.3 mm. Yellowish in color with brown pigment in liver and around mouth parts; small patches of russet on legs, mainly on carpi and meri, and on fourth and fifth abdominal segments; branching black chromatophores on eyestalks, on mouth parts, on rostrum, around heart, on postero-lateral part of carapace and between segments of abdomen except first and second, and sixth and telson.

Covered with hairs which are especially dense on distal parts of periopods. Front wide, with a broad tooth near junction of front and sides of carapace; margined with coarse setae. Rostrum blunt, pointing downwards. Surface of carapace depressed in median anterior part; bearing rounded prominences dorsally. Eyestalks elongated. Sixth abdominal segment smaller than others; telson (Fig. 2, *B*) wider than long and usually bearing three plumose setae on posterior margin.

Antennule (Fig. 2, *C*) now composed of peduncle and two flagella: first segment of peduncle swollen to lodge statocyst; third with three long terminal

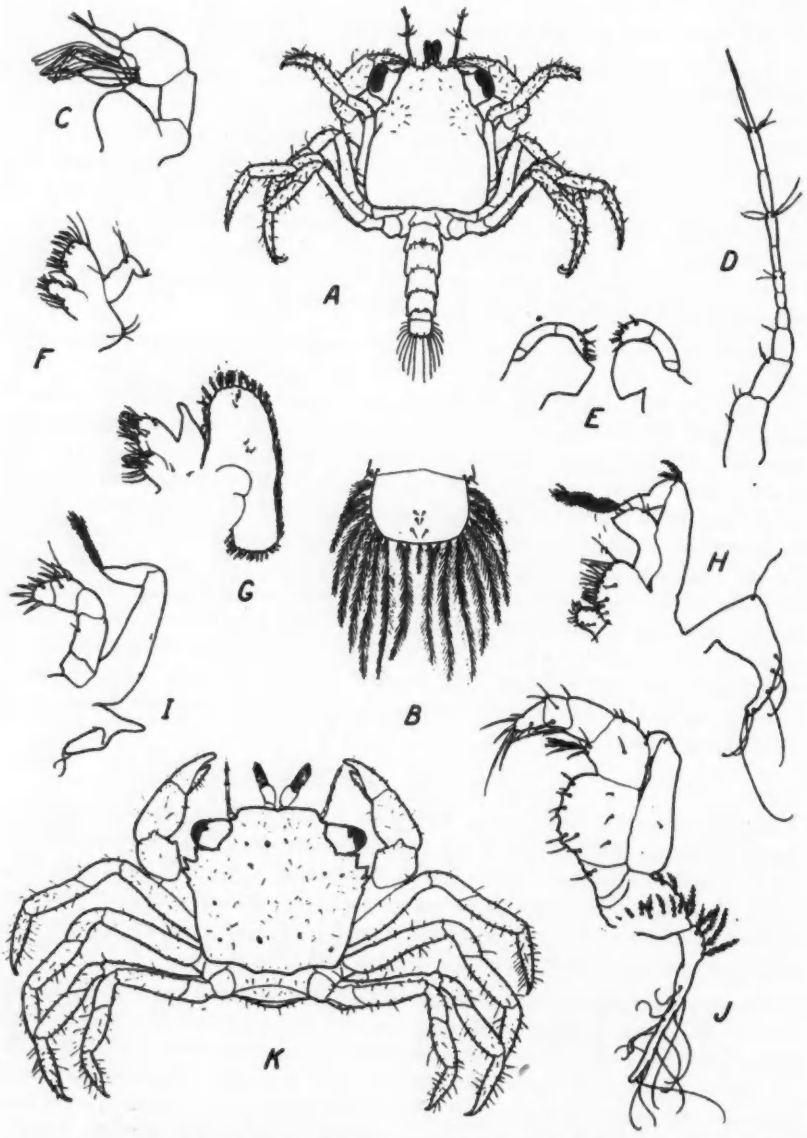

FIG. 2. *Lophopanopeus bellus* (Stimpson). *A, megalopa* × *15; B, telson* × *60; C, antennule* × *60; D, antenna* × *60; E, mandibles* × *60; F, maxillule* × *60; G, maxilla* × *60; H, first maxillipede* × *60; I, second maxillipede* × *60; J, third maxillipede* × *60; K, first young crab stage* × *15.*

setae, an unjointed flagellum bearing four setae and a segmented flagellum of three broad, short segments bearing aesthetes, and one longer, narrow, terminal segment with one bristle at tip. Antenna (Fig. 2, *D*) of about 11 segments: the proximal three enlarged to form a peduncle; and long bristles on distal segment, penultimate and fourth from tip. Mandible (Fig. 2, *E*) with two-jointed palp, bearing eight bristles on second joint: cutting surface of left mandible smoothly rounded, right coming to a point medially. Endopodite of maxillule (Fig. 2, *F*) decreased in size: now composed of a single segment with distal third twisted upon itself and bearing a few scattered bristles. Endopodite of maxilla (Fig. 2, *G*) somewhat degenerate, with only six soft plumose hairs on the proximal external margin, exopodite with a few setae on surface of scaphognathite as well as numerous marginal ones.

First maxillipede (Fig. 2, *H*) modified, as no longer a swimming appendage: endites of protopodite lobed and fringed with bristles; segmentation of endopodite lost, but a few small setae present distally; exopodite of two segments, the first with two soft hairs terminally, second with four; epipodite well developed. Second maxillipede (Fig. 2, *I*) has a five-jointed endopodite, with strong spines on terminal segments; exopodite two-jointed with five terminal hairs; small epipodite and a lateral projection that develops into a gill in next stage. Third maxillipede (Fig. 2, *J*) resembles that of adult, with flattened and swollen merus and ischium, margined with wide teeth on inner side; epipodite large, but exopodite not well developed. Chela stout, with large spine, the tip of which is bent medially, on ischium. Dactylus of fifth periopod with three slightly specialized setae, but not comparable to those found in some other families. Endopodites of first four pairs of pleopods have three hooked setae on distal internal margin; endopodites of fifth pair of pleopods (uropods) not developed; exopodites with 16, 16, 15, 13 and 8 (Fig. 2, *B*) long plumose setae.

First Young Crab Stage (Fig. 2, *K*)

Carapace about 1.7 by 2 mm. Similar in color to megalopa, with russet chromatophores on walking legs, giving a striped appearance when observed from above. A black chromatophore above heart, and on sternum at base of each cheliped; on ischium of third maxillipede is a bright orange spot, which is characteristic of the species.

Carapace with a broad frontal region, slightly indented medially, and also on lateral margin above eyes; three large teeth and one small one on lateral margin. Surface of carapace covered with minute hairs and scattered, longer setae.

Antennule like that of megalopa except that internal flagellum now composed of two segments, and outer of several indistinct joints with sensory hairs and three without aesthetes. Antenna similar, but more elongated. Now three segments on palp of mandible, second one bearing two setae and third 9-11. Terminal setae only, on endopodite of maxillule. Basipodite of maxilla elongated and exopodite broader with a number of setae on surface.

Plumose setae on inner margin of endopodite of first maxillipede and number increased on distal margin; epipodite larger. More setae on second maxillipede; gill developed. Merus and ischium of third maxillipede flatter, with sharp teeth on medial and distal margins.

Family Pinnotheridae

Hyman states, in reference to the family Pinnotheridae, that the complete history of metamorphosis has not been followed in any species. In his paper (5), he incorporates the work done on the group by authors previous to 1925 and adds descriptions of the early stages of two *Pinnotheres* and a *Dissodactylus* from the Atlantic coast of North America. He found a lack of uniformity in structure and in the number of zoeal stages. When the zoeae described by Aikawa (2) as "*Dissodactylozoea*" and "*Pinnozoea*" are assigned to their correct parental species, some will probably be the larvae of *Pinnotheres*, although superficially the larvae of *P. taylori* Rathbun resemble "*Grapsizoea brevispinosa*".

Lebour (9) describes the only *Pinnotheres* megalopa that has as yet been identified. This megalopa, *P. veterum* Bosc., differs in several respects from that of *P. taylori* described below.

Pinnotheres taylori Rathbun

A berried female, apparently of this little known species, was obtained from a transparent tunicate, March 16, 1933. Straw-colored, with bright orange eggs visible through the abdominal wall. Eggs in early stage of development and did not hatch until the first week in May; mass changing in color gradually to brown, and in size from 0.35 to 0.42 mm. Larvae when hatched covered with a spineless cuticle, shed with appearance of first zoea.

Two zoeal stages and one megalopal. First adolescent stage emerged four weeks after hatching in the laboratory. Zoeae cream-colored, with dark brown chromatophores at base of dorsal spine, around eyestalks, at base of antennules, on mandibles, on carapace postero-laterally, about the centre of basis of first maxillipedes and at junction of all segments of abdomen.

First zoea (Fig. 3, *A*) about 1.3 mm. long and 1.0 mm. between tips of spines. Rostral and dorsal spines rather short and blunt; laterals missing, but a tooth at postero-lateral margin of carapace, which is large and covers all except tips of swimming maxillipedes. Abdomen (Fig. 3, *B*) composed of five segments and telson; with sub-equal lateral protuberances on second and third segments; fourth and fifth segments somewhat swollen laterally, sides of telson (Fig. 3, *C*) parallel to one another, and no lateral spines, but surface covered with minute hairs, grouped in threes and fours, concentrated on telson forks, (which are very sharp pointed) so as to make them appear finely spined; six internal setae.

Antennule (Fig. 3, *D*) typical. Antenna (Fig. 3, *E*) small, with a short spinous process of protopodite, a swollen area (endopodite), and no exopodite. Mandibles like *Lophopanopeus bellus* but cutting surface rougher. Maxillule

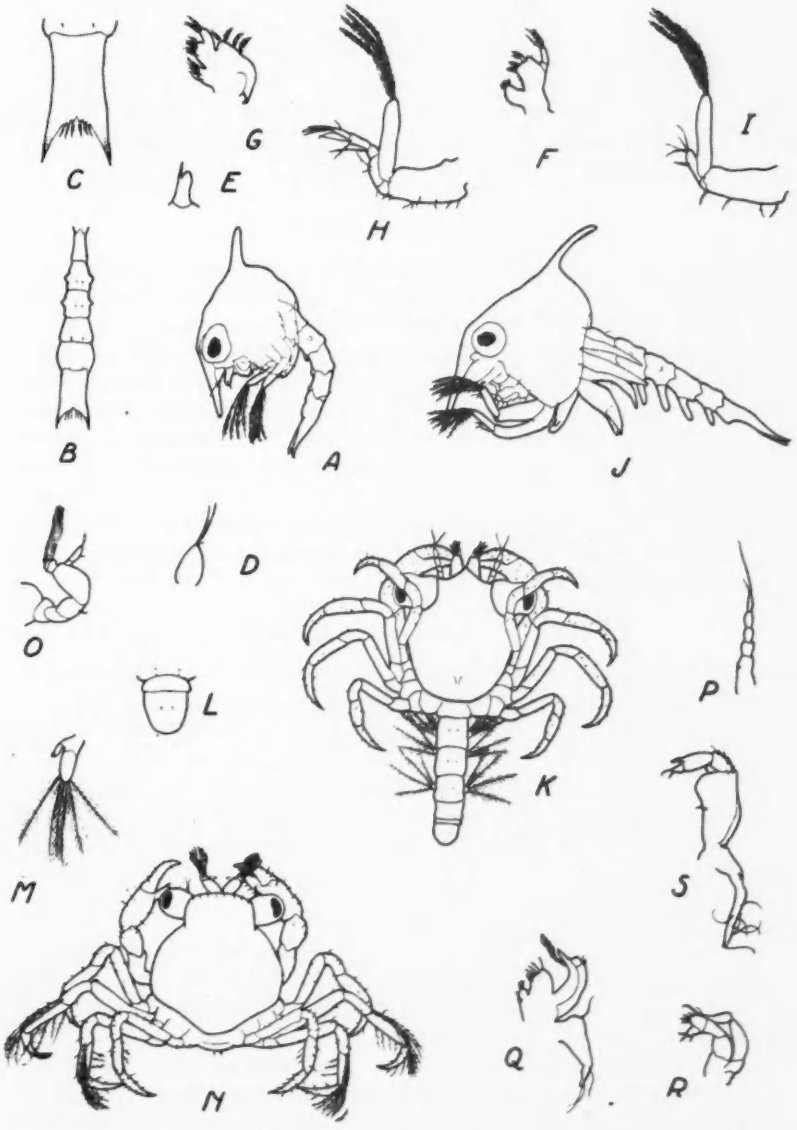

FIG. 3. *Pinnotheres taylori* Rathbun. *A, first zoea* × *30; B, abdomen* × *30; C, telson* × *60; D, antennule* × *60; E, antenna* × *60; F, maxillule* × *60; G, maxilla* × *60; H, first maxillipede* × *60; I, second maxillipede* × *60; J, second zoea* × *30; K, megalopa* × *30; L, telson* × *60; M, fourth pleopod* × *60; N, first young crab stage* × *30; O, antennule* × *60; P, antenna* × *60; Q, first maxillipede* × *60; R, second maxillipede* × *60; S, third maxillipede* × *60.*

(Fig. 3, *F*) usually with five spines on coxopodite and basipodite, and four on distal segment of the two-jointed endopodite. Endites of protopodite of maxilla (Fig. 3, *G*) bear six setae, endopodite three and exopodite four.

Exopodite of first maxillipede (Fig. 3, *H*) with 4 plumose setae; basis with 8 bristles; and endopodite with 1, 2, 1, 2 and 5. Second maxillipede (Fig. 3, *I*) correspondingly with 4; 4; and 0, 5 setae. Rest of thoracic appendages hidden beneath carapace; indication of two parts of third maxillipede (exopodite very small) and chela of first periopod. Anlages of four pairs of pleopods.

Second Zoea (Fig. 3, *J*)

Length about 1.6 mm. and 1.3 between tips of spines. Several setae on lateral part of carapace and two dorsally. Abdomen still of five segments and telson, and no additional internal setae.

Lateral extension of antennule indicative of endopodite of megalopa; aesthetes increased in number and in two rows. Endopodite of antennae enlarged, so longer than protopodite. Mandible with cutting surface divided into numerous small teeth. Plumose hair on external margin of protopodite of maxillule. More setae on maxilla, and three plumose projections on posterior part of scaphognathite.

Exopodite of maxillipedes with six swimming setae. Thoracic appendages becoming fully developed before zoea metamorphoses into megalopa. Pleopods greatly elongated but only four pairs.

Megalopa (Fig. 3, *K*)

Differs from *P. veterum* in presence of a small rostrum, a dorsal spine and a rudimentary sixth abdominal segment. Mustard-yellow in color, with transparent periopods. Black chromatophores on median part of carapace just posterior to eyes, and behind dorsal spine; also on each side of dorsal spine and between abdominal segments. On each segment (except dactylus of all but cheliped) of every leg is at least one spot of russet, usually medially placed. Ventrally, a black spot at bases of antennules and antennae, on mandibles, and at junction of chelipeds to sternum; russet at proximal junctions of other periopods. Carapace about 0.7 by 0.5 mm., with a small blunt spine on median posterior region.

Antennule large, with well developed peduncle, with distal segment especially elongated, and flagella rather small, with few setae. Antenna of about six segments; the distal one with one long and one short bristle, and the penultimate, one long seta. Apparently no palps on mandibles. Endites of protopodite of maxillule bear short, stiff spines on inner margin and one seta on external; endopodite rudimentary. Coxopodite of maxilla with six long plumose setae, and basipodite about nine short ones; endopodite quite degenerate, and exopodite with 20 setae around margin.

Setae few on first maxillipedes: four or five on each endite of protopodite, three on shrivelled endopodite, two on exopodite and epipodite. Second maxillipede with poorly developed exopodite, bearing three short setae

terminally; dactylus of endopodite inserted on proximal lateral margin of propodus, instead of terminally. Exopodite of third maxillipede small, with only one distal seta: merus and ischium fused, and dactylus placed in position similar to that of second maxillipede; epipodite small. Chela large and strongly developed. No setae on dactylus of last pair of periopods as is usual in Brachyrhyncha, but which seem to be lacking in all the Pinnotheridae. Only four pairs of pleopods, as none on small sixth abdominal segment (Fig. 3, *L*): endopodite of each pleopod (Fig. 3, *M*) bears two short curved setae, and exopodite, six very long plumose hairs.

FIRST YOUNG CRAB STAGE (FIG. 3, *N*)

Small, carapace about 0.75 by 0.7 mm. Straw-colored, with periopods a shade deeper in color than body. Front broad, and fringed with about 10 short setae; no trace of dorsal spine of megalopa. Young crab able to swim freely—as does adult—by means of long plumose setae on distal segments of third and fourth pairs of legs.

Antennule (Fig. 3, *O*) large, with distal segment of peduncle swollen and elongated. Antenna (Fig. 3, *P*) of about five segments, with two bristles at tip. Maxillule and maxilla like megalopa. First maxillipede (Fig. 3, *Q*) has a few more setae than megalopa and is quite large. Second maxillipede (Fig. 3, *R*) little changed. Merus-ischium of third maxillipede (Fig. 3, *S*) swollen and flattened distally and dactylus inserted more distally than in megalopa; setae on distal part of epipodite. Chela large and sparsely covered with setae, as are all periopods. Swimming setae present on distal part of carpus and propodus of third and fourth legs.

Family Grapsidae

Hyman (6) found the larvae of the family Grapsidae quite uniform in structure. Aikawa (1), however, observed that the larvae of some of the Japanese forms did not agree with the earlier described types, either in the absence of lateral spines on the carapace, or in the length of the antennae. The development of the two Grapsoid species in British Columbia is similar to that of related forms in Japan. The megalopae that have been described, (with the exception of the first one attributed by Cano to *Pachygrapsus marmoratus* (Fabricius), and which has a large rostrum), are very like those obtained by the writer. There are no sensory setae on the last periopods of the megalopae in Cano's drawings, which is perhaps an oversight, as they are present in most Brachyrhyncha. Rathbun (11) gives figures of megalopae, which are designated as "Grapsoid", *Pachygrapsus crassipes* (?) and *Sesarma magdalensis*, all of which are of the same type as those of *Hemigrapsus* described below.

The first zoea of *Hemigrapsus longitarsis* (Miers), as described by Aikawa (1), is similar to the larvae of *H. nudus* (Dana) and *H. oregonensis* (Dana). It is slightly smaller and there are two extra setae on the endopodite of the first maxillipede. The distribution of the setae differs on the joints of the second maxillipede.

Hemigrapsus nudus (Dana)

Berried females found commonly in April and May at Departure Bay, British Columbia; rarely in June, but reported by Bovard and Osterid (3) to be carrying eggs in June and July at Friday Harbour, Washington. Freshly laid egg mass purplish-black, changing to gray when ready to hatch. Increase in size from 0.38 to 0.45 mm. in diameter.

Five zoeal stages and one megalopal; prezoeal almost disappeared; embryonic cuticle, shed on hatching, has no embryonic spines on antennae or telson, but spines and setae invaginated upon themselves typically.

First zoea (Fig. 4, *A*) about 1.65 mm. in length and 1.2 mm. between tips of spines. Very transparent, with a yellow tinge to body and dark chromatophores: pigment spots around eyestalks, one on carapace between eyes, on labrum, on bases of antennules and antennae, on mandibles, on distal part of bases of first maxillipedes, and at bases of dorsal and lateral spines; some pigmentation at joints of each segment of abdomen and around proximal part of intestine. On dorsal spine and also on fourth abdominal segment, red chromatophores. Rostral spine and forks of telson tinged with violet and lateral spines with russet.

First zoea (Fig. 4, *A*) stout, with all carapacial spines well developed. Postero-lateral margin of carapace bears fine teeth and setae. Abdomen (Fig. 4, *B*) of five segments and telson; large knobs on second abdominal segment and smaller ones on third. External spines lacking on telson, but tips of fork cut internally into fine teeth and a row of minute spines on dorsal surface of each cornua; six internal setae.

Antennule (Fig. 4, *C*) tubular-conical, usually with two long aesthetes and one short seta. Process of protopodite of antenna (Fig. 4, *D*) bears two rows of spines distally, is longer than exopodite, and reaches to about distal third of rostral spine; exopodite with three setae about one-third from tip, which is very minutely spined. Mandibles (Fig. 4, *E*) bilobed, each with a broad denticulated incisor process, and a smaller, more sharply divided molar: left mandible with additional tooth on ventral side. Endite of coxopodite of maxillule (Fig. 4, *F*) usually with five plumose setae, basipodite six, endopodite one and five. Endite of coxopodite of maxilla (Fig. 4, *G*) bilobed with six plumose setae, basipodite with seven; endopodite four and narrow exopodite five, soft, densely plumose setae.

Nine bristles on basis of first maxillipede (Fig. 4, *H*), 4 swimming setae on exopodite, and 2, 2, 1, 2 and 5 setae on joints of endopodite. Second maxillipede (Fig. 4, *I*) with 4 setae on basis, 4 natatory on exopodite and 0, 1 and 6 on endopodite. Rudimentary thoracic appendages present as small lobes under carapace.

Second to Fifth Zoeae

All zoeal stages similar in coloration; chromatophores enlarge and branch, and spines become almost colorless as zoea develops. Second zoea (Fig. 4, *J*) about 1.9 mm. long and 1.6 mm. between tips of spines; third (Fig. 4, *K*)

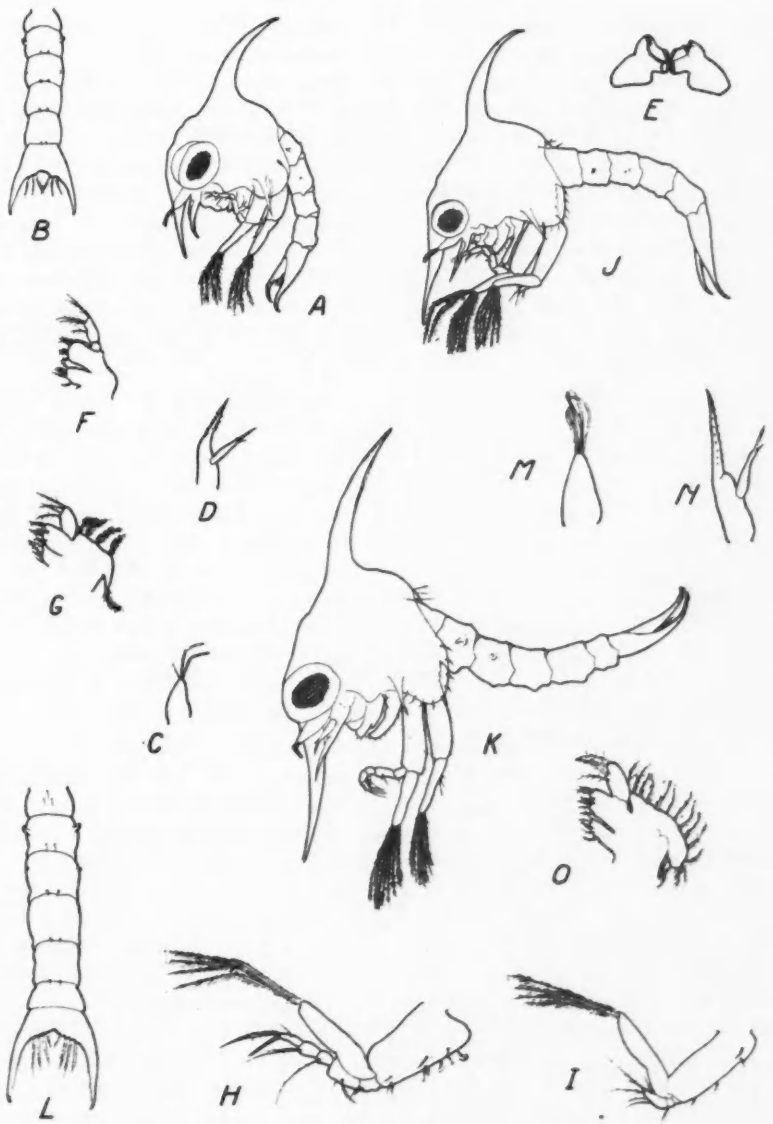

FIG. 4. *Hemigrapsus nudus* (Dana). *A, first zoea* × *30; B, abdomen* × *30; C, antennule* × *60; D, antenna* × *60; E, mandibles* × *60; F, maxillule* × *60; G, maxilla* × *60; H, first maxillipede* × *60; I, second maxillipede* × *60; J, second zoea* × *30; K, third zoea* × *30; L, abdomen* × *30; M, antennule* × *60; N, antenna* × *60; O, maxilla* × *60.*

2.2 and 2.2 mm.; fourth (Fig. 5, *A*) 2.6 and 2.8 mm.; and fifth (Fig. 5, *B*) 3.3 and 3.5 mm. Length of spines as compared to animal as a whole varies with different stages; lateral spines increase very little and in fourth and fifth stages are bent downwards and hardly reach margin of carapace. Marginal hairs increase greatly in size, until in last zoeal stage they are long and plumose; hairs also appear on mid-posterior margin of carapace. Abdomen composed of six segments in second zoea but seventh appears between last segment and telson in third zoea (Fig. 4, *L*); knobs present in all stages on second and third segments; long setae on first segment: one in second stage, three in third, five in fourth and seven in fifth. An additional pair of setae appear internally in telson of third zoea and again in fourth, so that in last two stages five pairs of setae are present. Slight indication of position of pleopods by small swellings on second to fifth abdominal segments of third zoea; in fourth stage increase in size and a small pair appear on sixth segment; in fifth, these anlages long, tubular structures with indications of three parts.

Antennules (Fig. 4, *M*) of second to fourth zoeae similar to first, except that the number of sensory setae increase with age; in fifth zoea (Fig. 5, *C*) indications of adult shape seen: a swelling at base for lodging of statocyst, and a lateral projection, the flagellum (endopodite) distally. Last two zoeae have aesthetes arranged in two series, terminal and sub-terminal. Antennae increase little in size with development, consequently their relative length as compared with rostral spine varies. First indication of flagellum seen in third zoea (Fig. 4, *N*) where a slight swelling occurs between exopodite and spinous process of protopodite; at next moult it reaches nearly to tip of exopodite and finally (Fig. 5, *D*) is sub-equal to protopodite process; indication of segmentation can be seen shortly before metamorphosis to postlarva; spines on tip of exopodite increase in size but never become very clear.

Mandibles of second and third stages like those of first; fourth and fifth have one tooth with a very broad cutting surface; and a small protuberance on fourth zoea mandible enlarges to form the bare, unjointed palp of fifth stage. Setae and spines on endites of maxillule increase slightly in number; appendage becomes stronger but changes little in shape: endopodite (Fig. 5, *E*) of all stages bears one and five setae on its segments; a very densely plumose seta present on external margin of protopodite of all but first zoea and a bare one in addition in the last three zoeae. Fringe of setae on segments of protopodite of maxilla (Figs. 4, *O* and 5, *F*) becomes denser as zoea develops, exopodite enlarges and becomes more plate-like, margined with increasing numbers of densely plumose soft hairs; endopodite with 4 setae throughout.

Swimming setae on tips of exopodites of first maxillipedes are 6 in second stage, 8 in third, 10 in fourth and 12 in fifth; usually 9 setae on basis and 2, 2, 1, 2 and 5 on segments of endopodite, but additional ones found on last three segments of later stages. Setae on exopodites of second maxillipedes correspond in number to those of first; usually 4 setae on basis but these may be reduced to 3 in later zoeae; all have 0, 1 and 6 setae on endopodite. There is an indication of the division into the two rami of the rudimentary third

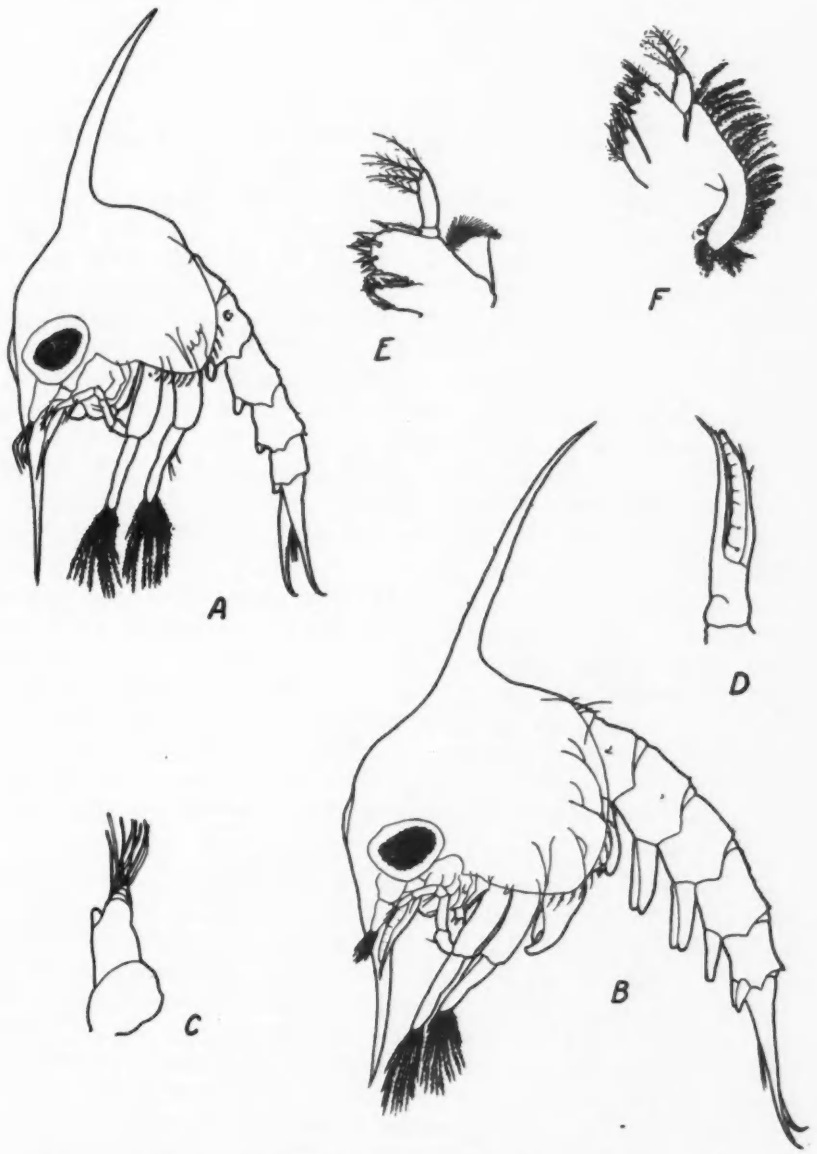

FIG. 5. *Hemigrapsus nudus* (Dana). *A, fourth zoea* × *30; B, fifth zoea* × *30; C, antennule* × *60; D, antenna* × *60; E, maxillule* × *60; F, maxilla* × *60.*

maxillipede and an indentation at the tip of the first periopod in the third zoea. The segmentation of thoracic appendages can be seen through the enveloping membranes in fifth zoea.

MEGALOPA (FIG. 6, *A*)

Resembles those described and figured by Rathbun (11). About 3.6 mm. in length, and carapace 1.8 to 1.5 mm. Yellowish in color, with scattered black chromatophores: a yellow patch shows through transparent carapace just anterior to gastric mill and another posterior to heart, liver a light brown; dark branching chromatophores on eyestalks, on carapace above gastric region, beneath heart, surrounding intestine, and on joints between all abdominal segments with the exception of the last two; on appendages: on bases of antennae, on mandibles, maxillae, second maxillipedes, coxopodites of chelae and distal part of merus, carpus and propodus and distal part of dactylus of third and fourth legs. Slight amount may be on proximal margin of carpus of fifth leg. Carapace smooth, undulating, with a wide front, the centre of which is depressed where the short, downward-pointing rostrum occurs. Abdomen composed of six segments and telson (Fig. 6, *B*), which is smoothly rounded and bears three or four short plumose setae on distal margin.

Antennule (Fig. 6, *C*) very like that of adult: large swollen base, bearing a peduncle of two segments, the distal one with an unsegmented and a segmented (with sensory hairs) flagellum. Antenna (Fig. 6, *D*) composed of about 11 segments, the distal 3 bearing a number of long bristles. Mandible (Fig. 6, *E*) with strong cutting endite, a two-jointed palp, the second segment of which bears 8-10 short stiff setae. Maxillule (Fig. 6, *F*) like that of adult, with rudimentary endopodite. Endopodite of maxilla (Fig. 6, *G*) now bare of setae and atrophied, but other parts increased in size and well armed with spines and setae.

First maxillipede (Fig. 6, *H*) with well developed endites of protopodite armed with a number of stiff setae; endopodite shrunken and with no definite segmentation; exopodite with four terminal plumose hairs and two at distal margin of first joint; and large epipodite. Second maxillipede (Fig. 6, *I*) has endopodite with five definite segments, terminal ones bearing stiff hairs, and a small epipodite is present. Third maxillipede (Fig. 6, *J*) of typical brachyuran type, with well developed endopodite, exopodite and epipodite. A spur on distal posterior point of propodus of second to fourth legs and three sharp teeth on posterior margin of dactylus. Dactylus of fifth leg (Fig. 6, *K*) bears three long setae, two of which have "combs" in addition to spinules. No hooks on coxae of periopods. Five pairs of pleopods; endopodites of first four pairs each with two stout curved setae and exopodites with 18-20 long plumose hairs; uropods with 9 or 10 long hairs on exopodites.

FIRST YOUNG CRAB STAGE (FIG. 6, *L*)

Carapace square, about 2 mm. in length and breadth. Front of two curved lobes, unlike the straight frontal margin of adult. Eyes large and extend

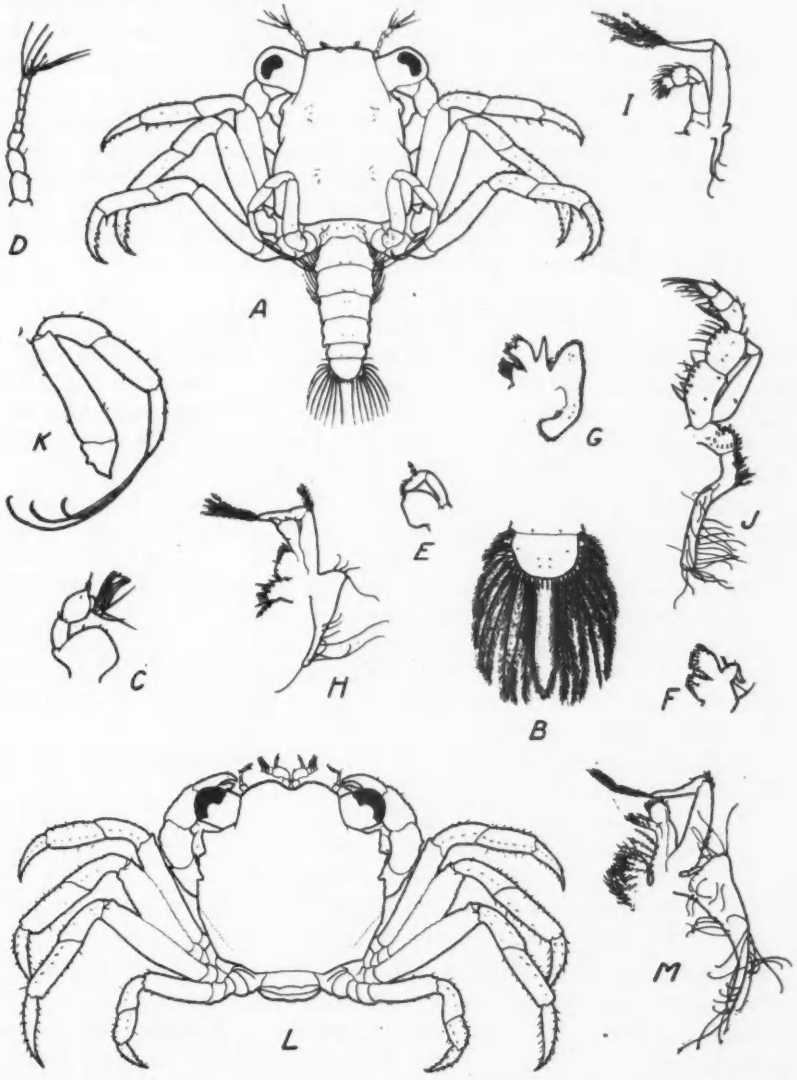

FIG. 6. *Hemigrapsus nudus* (Dana). *A, megalopa* × *15; B, telson* × *30; C, antennule* × *30; D, antenna* × *30; E, mandible* × *30; F, maxillule* × *30; G, maxilla* × *30; H, first maxillipede* × *30; I, second maxillipede* × *30; J, third maxillipede* × *30; K, fifth periopod* × *30; L, first young crab stage* × *15; M, first maxillipede* × *30.*

past sides of carapace, which are parallel for over half their length; three distinct lateral teeth on carapace, the margins cut by minute denticulations. Fine tubercles on carapace and periopods, arranged in definite rows on posterior lateral part of carapace and on chelipeds. All periopods beset with fine hairs, which is unexpected considering the specific character of the mature *nudus*. Usually yellowish in color with varying amounts of black pigment on distal parts of third and fourth legs, and abdomen.

The appendages, with the exception of the first maxillipede and the shrivelled pleopods, are so similar to those of the megalopa that a description of them seems unnecessary. First maxillipede (Fig. 6, *M*), however, shows in endopodite the beginning of the modification to the unique type found in the adult, where endopodite is grooved and tip split so that exopodite lies closely applied and almost surrounded by endopodite.

Hemigrapsus oregonensis (Dana)

Berried females were found near Vancouver, on March 17, 1930, which is some weeks earlier than they have been obtained at Departure Bay, where they hatch from middle of May until August. Early egg mass varies from purplish-black to russet, and when ready to hatch is a light brown. Eggs increase in size from about 0.33 to 0.40 mm.

Zoeal stages five, and megalopal one. First young crab stage has been twice reared, taking four and five weeks. Fourth young crab stage appeared after four and one-half weeks.

The stages correspond closely to those of *H. nudus*. The main distinguishing characters are the smaller size, and absence of a knob on third abdominal segment (Fig. 7, *A*) of the zoeae, and of setae on the posterior margin of the telson (Fig. 7, *B*) in the megalopa. Color seems to be identical in the two species. Dorsal and rostral spines, and abdomen of *H. oregonensis* more slender than *H. nudus*.

Length of different stages (Fig. 7, *C-G*) and distance between tips of spines as follows:

First zoea	1.3 mm. and 1.1 mm.
Second zoea	1.6 mm. and 1.6 mm.
Third zoea	2.0 mm. and 2.0 mm.
Fourth zoea	2.4 mm. and 2.5 mm.
Fifth zoea	2.7 mm. and 2.5 mm.

The development of the zoea is so like that of *H. nudus* that a detailed description seems superfluous. Appendages differ only in size.

Megalopa (Fig. 7, *H*) about four-fifths the size of *H. nudus*, and carapace somewhat narrower in proportion. Length about 2.9 mm. and carapace 1.5 by 1.1 mm. No plumose setae on smoothly rounded posterior margin of telson. Appendages naturally somewhat smaller but in form resemble *H. nudus*. First maxillipede differs slightly; endopodite smaller in proportion, and endites of protopodite bear longer setae on a more rounded margin.

First four pairs of pleopods have 14-17 long plumose setae on exopodite; uropods usually have a soft plumose seta on protopodite and eight or nine on exopodite.

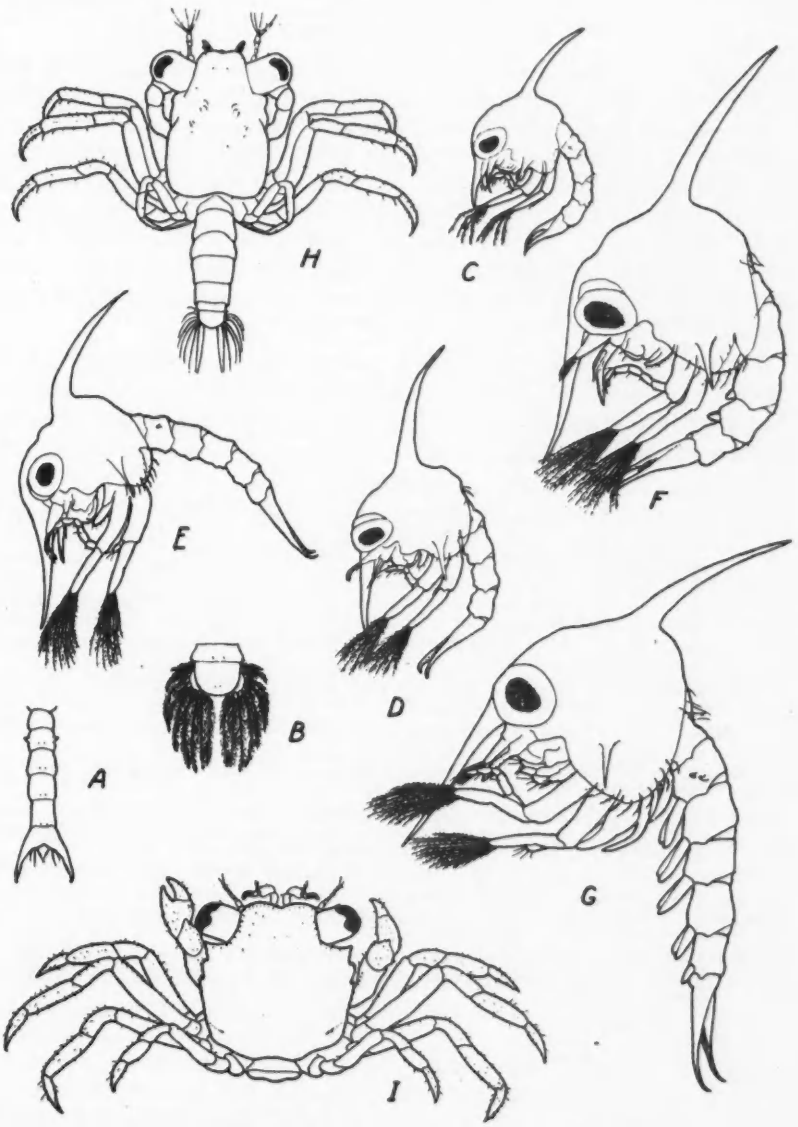

FIG. 7. *Hemigrapsus oregonensis* (Dana). *A, abdomen of first zoea × 30; B, telson of megalopa × 30; C, first zoea × 30; D, second zoea × 30; E, third zoea × 30; F, fourth zoea × 30; G, fifth zoea × 30; H, megalopa × 15; I, first young crab stage × 15.*

First young crab stage (Fig. 7, *I*) also closely resembles that of *H. nudus*. Carapace 1.6 by 1.6 mm. Chelipeds and walking legs not quite as stout as those of the related species.

Acknowledgments

This study of the development of Brachyura was commenced in 1930 at the Pacific Biological Station, and continued at the University of British Columbia and the University of Toronto. I am indebted to Dr. W. A. Clemens, Dr. C. McLean Fraser and Dr. E. M. Walker for their supervision of the work and many kindnesses. Dr. Alfreda Berkeley Needler suggested the problem and gave me much valuable advice. Professor G. J. Spencer gave suggestions for the preparation of the material for description. I wish also to thank the staff members of the Biological Station for their co-operation and help in obtaining material and in suggesting methods of coping with numerous difficulties of the experimental work.

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POST EMBRYOLOGICAL DEVELOPMENT OF EPHEMEROPTERA (MAYFLIES). EXTERNAL CHARACTERS ONLY[1]

By F. P. Ide[2]

Abstract

The development of external structures is followed from hatching to maturity. The life histories of two species *Stenonema canadense* Wlk. and *Ephemera simulans* Wlk. are described in detail and less complete accounts given of nine others.

Each moult results in a change in the number of segments in the caudal filaments and on this basis it has been possible to determine the number of instars. In *Stenonema canadense* the number was found to be between 40 and 45, and in *Ephemera simulans* about 30. Segments are added to the caudal filaments of the former at each moult in the following way: one in each of the first two moults, two in each of the next three, three in the next and four at each subsequent nymphal moult. In the change from nymph to subimago distal segments are dropped in *Ephemera* and probably in *Stenonema* also.

Mouth parts are very different in the newly hatched than in the full grown nymph as described for *Stenonema canadense.*

A definite case of a hypermetamorphosis of the tarsus and its claws appears in the life history of *Epeorus humeralis* and *Iron pleuralis.*

Gills are absent in the first instar in all the species studied. In some species they appear simultaneously on all the gill bearing segments at the first moult. In others they appear on segments five and six only at this moult, those of the other segments appearing only after several moults. The internal or secondary ramus of the gills appears much later in the nymphal life. The ultimate shape of the gill is influenced in some cases by the fact that the gills of segments five and six are segmented or unsegmented.

In *S. canadense* the wing pads make their appearance in about the fifteenth from the last instar and the claspers and external genitalia of the male are apparent in about the eighth from the last instar.

At each moult there is some structural change in the nymph adapting it to the environment. The environmental relation is being constantly changed by increase in size of the organism, thus necessitating these adaptations to preserve an equilibrium.

Introduction

There is relatively little known of the younger stages of Ephemerid nymphs and a study of these is important not only to a better knowledge of the insects themselves but of their distribution and ecology.

Lubbock (1864 and 1867) describes very fully the development of *Cloeon dimidiatum* but has missed at least one of the early instars and misinterpreted the way in which segments are added in the caudal filaments. Joly (1872) describes in brief some of the changes taking place in the nymphs of *Ephoron* (*Palingenia*) *virgo*. (See also Joly (1876).) Vayssiere (1882) describes the development of *Ecdyonurus* (*Heptagenia*) *venosus* (*longicauda*) describing nine stages which, however, do not correspond to instars. Lestage (1921) divides the nymphal life into three stages, stade larvulaire, stade larvaire and stade larva-nymphal. Murphy (1922) has reared *Baetis posticatus* Say and finds that there are 26 instars in this species. Gros (1923) in a study of *Ecdyonurus forcipulus* Koll. has described more stages than Vayssiere, basing the separation mainly on the growth of the gills. Wiebe (1926) describes the first three instars of *Hexagenia bilineata* Say and Neave (1932) the first instar of *Hexagenia limbata occulta* Walk.

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In the present paper nymphal stages of 11 species of mayflies commonly found in the upper reaches of trout streams of Ontario are described. The species are:

1. *Ephemera simulans* Walker;
2. *Stenonema canadense* Walker;
3. *Stenonema fuscum* Clemens;
4. *Stenonema tripunctatum* Banks;
5. *Heptagenia pulla* Clem;
6. *Heptagenia hebe* McD.;
7. *Epeorus humeralis* Morgan;
8. *Iron pleuralis* Banks;
9. *Isonychia bicolor* Wlk.;
10. *Ephemerella subvaria* McD.;
11. *Leptophlebia debilis* Wlk.

The life histories of the first two species mentioned were worked out fairly completely, but even in these all the instars were not determined. In both cases, however, the method of addition of segments in the caudal filaments and antennae was determined. It was found that each moult results in a change in the segmentation of the caudal filaments. The number of instars through which the full-grown nymph has passed may be estimated fairly closely by an examination of the caudal filaments and could be definitely determined in this way if there were not a fusion and obliteration of some of the segments basally. In *Ephemera simulans* the number of instars was estimated at about 30 and in *Stenonema canadense* at between 40 and 45.

For other species listed much less complete accounts are given, especially for *Ephemerella subvaria* and *Leptophlebia debilis*. The description is of the eggs and first instar of the former, and the gills of the latter.

The material was collected from the Mad and Pine rivers, tributaries of the Nottawasaga river, in Dufferin and Simcoe counties, Ontario, in connection with an ecological study of mayflies of these streams conducted during the summers of 1930, 1931 and 1932.

All the species herein treated have already been adequately described in the adult and full-grown nymphal state; references to these descriptions are given in the case of each species.

Method

Nymphs were collected periodically in the stream and examined under a compound microscope in the following way. A film of water was spread over a microscope slide and nymphs of one species placed dorsal side up in rows to facilitate the taking of measurements. When thus arranged the nymphs were covered with a cover slip and water was introduced, care being taken to exclude all air bubbles. The temporary mount thus made was placed on a larger piece of plate glass for convenience in handling under the objective of the microscope. Measurements were made using a squared whipple disc in a 10× Spencer ocular. For the later instars in which the mesothoracic wing pads are growing out rapidly relative to the growth of the nymph as a whole, the length of these pads was measured and also the length of the seventh abdominal tergum. The length of the wing pad was then divided by the length of the seventh tergum of the same individual to give a factor x.

By this means the differences in sizes of nymphs at the same instar were compensated for. As the wing pads grow out, not evenly, but by sudden increases in length at each moult, frequency diagrams made by plotting values of x as abscissas and the number of individuals as ordinates, gave a separation of the later stages into groups. Each group, it was shown, did not always represent one instar, since in one case it was found that there was little increase in length of the wing pads, but that in male individuals the instars could be easily separated by the change in size of the claspers which are growing out at the same time as the wing pads.

It was found that the intermediate stages could be roughly grouped by making a count of the segments in the antennae. Other structures which showed metamorphosis at each moult were the gills, and in the case of *Ephemera simulans*, the mandibular tusks. Very minute changes in morphological structure were found to be very constant and to indicate true instars.

The very early stages were never taken in collections in the stream owing to their small size (less than a millimetre). These were secured by capturing female adults on their ovipositing flight and relieving them of their egg masses which were set to incubate in fresh water in glass containers. Usually it was found that the eggs incubated under these conditions and hatched, even in the case of rapid water forms. With the rapid water forms, however, all individuals died either in the first or second instar. *Stenonema canadense*, and *Ephemera simulans* were reared further, up to the eighth or ninth instar.

Samples were taken from the rearing jars at intervals of two or three days and notes made on all the individuals contained in the sample, special attention being given to the changes in segmentation of the caudal filaments and of the antennae, the change in the number of spines on the femora and the appearance and subsequent increase in size of the gills. These structures were very sensitive indicators of change at moulting, especially the caudal filaments which, it will be shown in this paper, register every moult through which the individual passes.

Examination of the early instars was carried out under the compound microscope, using a 4 mm. Spencer objective and a 10× ocular, and examination of the older stages under a 16 mm. Spencer objective and a 10× ocular. The oil immersion was used to examine the mouth parts of the first and fifth instars of *Stenonema canadense*.

All drawings were made on cards ruled in quarter-inch squares and each is drawn to one of three scales of magnification, designated X, Y or Z, depending on whether the 16 mm., 4 mm., or oil immersion objective was used. The scales of magnification are given in Fig. 2,-13, and the magnification of each figure is mentioned in the explanation of the plate concerned. In any one figure drawings of structures having the same number were made from one individual (except in one or two instances), letters designating the separate structures.

Description of Instars

In the description of the instars of *E. simulans* and *Stenonema canadense* some characters have been tabulated to save repetition. These characters are omitted from the general accounts so that the data in the tables should be consulted as supplementary material.

Ephemera simulans Walker

The full grown nymph of *E. simulans* was described by Clemens (1915). This stream form, which may eventually be shown to be specifically different

TABLE I

DIAGNOSTIC CHARACTERS OF EARLY AND LATE INSTARS OF *Ephemera simulans*

Instar	Length in mm.			No. of segments in antennal flagellum (including terminal piece)	Apparent no. of segments in ant.	No. of segments in caudal filament including terminal piece	Gills	Setae on posterior border of femur
	No. of individuals	Average	Lower and upper limits					
1	(25)	0.79	(0.64–0.85)	3		5	Absent	1 (and hairs)
2	(27)	0.82	(0.72–0.91)	4		5.5	On Segs. 2-7	1 (and hairs)
3	(4)	0.94		5	4	6	On Segs. 2-7	2
4	(4)	0.99	(0.87–1.12)	6	5	7	On Segs. 2-7	
5				7	6	9	On Segs. 2-7	5
6				8	7	12	Gill 7, sec. ramus begun	6
7				9	8	16		12
8								
9				11	10			
?10				12	10			13
11				13	11			
12								
13				15	13			26
14- about 23								

	Length of mesothoracic wing pad of male, mm.	Distance between wing pads at base, mm.	Segments in male claspers	Length of male claspers
?Nonult	0.29			visible
Octult				visible
Septult	0.42	0.40	1	0.05 mm.
Sextult	0.42	0.50	1	0.10
Quintult	0.64	0.50	2	0.10
Quartult	0.74	0.50	3	0.22
Tertiult	1.10		3	0.34
Penult	1.36		3	0.53
Last	2.60		3	0.85

The length of the nymphs is measured from the front of the head to the tip of the abdomen excluding the length of the caudal filaments. Where the length is the average of a number of individuals the number measured is placed in brackets and also the upper and lower limits of the lengths. In recording the segments of the antennae the two basal segments are omitted but the terminal segment is included in the count throughout. Similarly in the count of segments in the caudal filaments the terminal segment is included. The arrangement of setae on the femur refers to the series on the posterior border only.

Some segments are fused basally in the antenna and caudal filament and in these cases the true number and the apparent number are listed.

from typical *E. simulans* of lakes, was present in countless numbers in the slower flowing reaches of the Mad river at Singhampton, Ontario, where its nymphs burrow in the gravel and marl of the bottom in the channel of the stream.

Eggs oblong, about .25 × .15 mm. (Fig. 1,–1). Surface of the egg reticulate as shown (Fig. 1,–1). The egg figured is one from which the nymph had hatched. Eggs extruded in two cylindrical masses, one from each oviduct.

First instar (Fig. 1,–2). Average length of 25 individuals .79 mm. with upper and lower limits of .85 and .64 mm. respectively. Head somewhat pentagonal, front margin forming two sides of pentagon; compound eyes rather less prominent than lateral ocelli and directly behind them; antennae composed of two thick basal segments and terminal flagellum in which are two segments differentiated and a terminal undifferentiated portion about as long as the second segment; hairs present subapically in these segments. Yolky enteron visible as an opaque body (stippled in figure); femora with one rather weak distal seta and two proximal hairs. Gills absent; three subequal caudal filaments, each of five segments of which the three basal are well differentiated and the others indistinct; rosette of spines present apically on Segment 1 only; Segments 2 and 3 armed apically with fine hairs.

Second instar, (Fig. 1,–3, 3a and 3b). Basal segment of antennal flagellum shorter and thicker than in first instar. Gills on Segments 2-7 inclusive as projections of the postero-lateral angles. The basal segment of caudal filament thickened, and shortened, the second segment better differentiated; a rosette of rather weak spines apically as on the basal segment; Segments 3 and 4 longer than in previous instar; Segment 5, the terminal piece more than twice as long as Segments 4 and 5 of the previous instar. The writer is inclined to think that this segment represents two undifferentiated segments; if this is so it means that the equivalent of a segment has been added in this instar to those formed in the previous instar.

Third instar, (Fig. 1,–4, 4a, 4b, 4c). The basal segment of antennal flagellum more elongate than in the previous instar, and probably the product of the two basal segments fused, making five segments in all; on the basal segment of the antenna adjacent to the flagellum a pair of very stout curved setae, which are present in this position in all subsequent stages. Femora each with a distal stout seta on posterior border and immediately proximad of it a weaker seta; gills elongate, uniramous; Gill 7 shown in Fig. 1,–4; on the lateral border of the gill in the distal third, a process, the first of the series of filaments which develop along the lateral border.

Caudal filaments (Fig. 1,–4b) have added one segment to the number already formed in the second instar, so that there are now six segments in all; basal segment much shortened and thickened.

Fourth instar, (Fig. 1,–5, 5a, 5b and 5c). Rostrum (Fig. 1,–5) developed as a shelf with anterior margin convexly curved; seventh gill of right side shown in Fig. 1,–5a. It is about twice as long as in the third instar, still

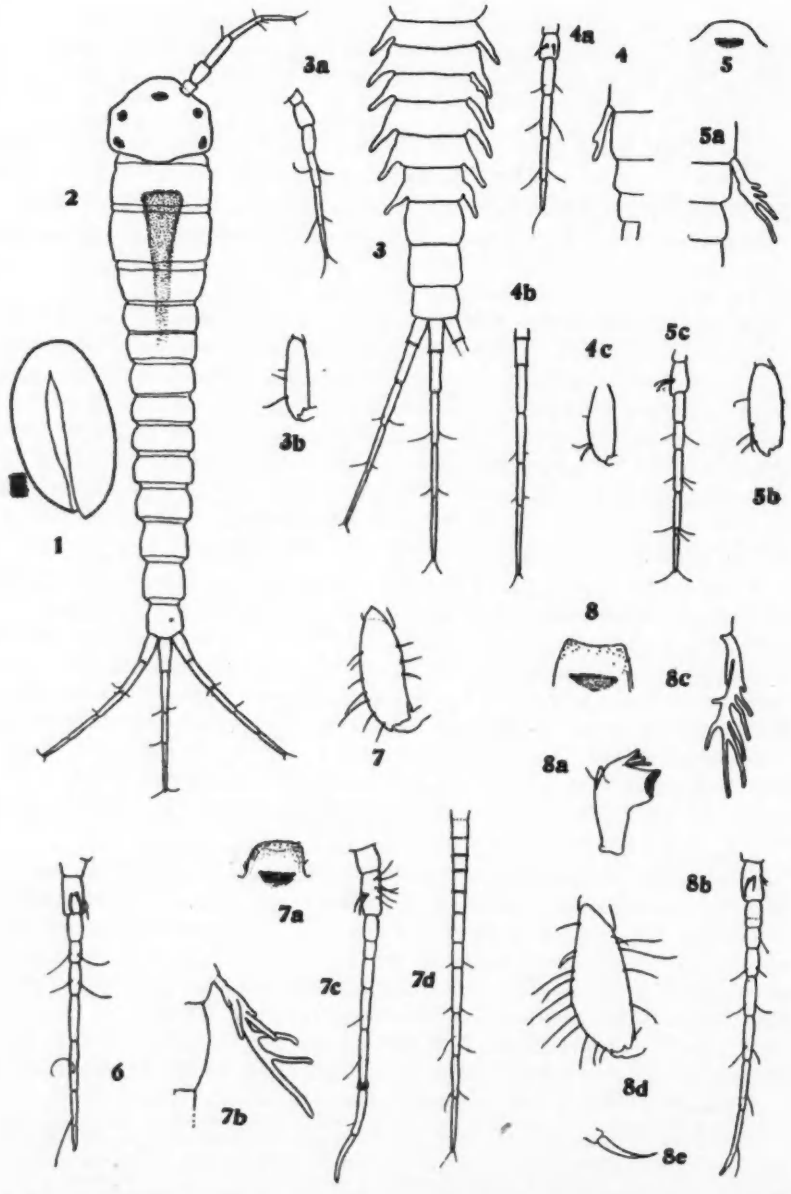

FIG. 1. *Ephemera simulans. All mag. Y. 1, Egg. 2, First instar. 3, Second instar, abdomen; 3a, antenna; 3b, femur. 4, Third instar, Gill 7; 4a, antenna; 4b, caudal filament; 4c, femur. 5, Fourth instar, rostrum; 5a, Gill 7; 5b, femur; 5c, antenna. 6, Antenna, fifth instar. 7, Sixth instar, femur; 7a, rostrum; 7b, seventh gill; 7c, antenna; 7d, lateral caudal filament. 8, Seventh instar, rostrum; 8a, mandible; 8b, antenna; 8c, seventh gill; 8d, hind femur; 8e, tarsal claw.*

uniramous, but with three lateral processes instead of one. The three basal segments of the caudal filaments now armed apically with a rosette of short spines.

Fifth instar (Fig. 1,–6). The rostrum is more truncate than in the fourth instar; caudal filaments with nine segments in flagellum, an addition of two to those present in the fourth instar. Gill 7 with five filamentous appendages but still uniramous.

Sixth instar, (Fig. 1,–7, 7a, 7b, 7c and 7d). At distal end of Segment 5 of the antenna is a thick sclerotized region, and in most individuals of this stage the antenna was broken off at this point. Usually the antennae were broken off at different points, one losing practically the whole flagellum and the other about one third of it. Rostrum (Fig. 1,–7a), more extended and truncate than in previous instar, and its surface roughened with numerous tubercles. Gill 7 more complex and with the beginning of the internal or secondary ramus, (Fig. 1,–7b). Caudal filaments show an addition of three segments in the flagellum, making 12, including the terminal piece.

Seventh instar, (Fig. 1,–8, 8a, 8b, 8c, 8d and 8e). Rostrum slightly emarginate. This condition is forecast in the previous instar figured where the new rostrum is visible through the old nymphal skin. Mandible (Fig. 1,–8a) with tusk well under way in development; tarsal claw long, scimitar-shaped and lacks pectinations.

Ninth instar, (Fig. 2,–9, 9a and 9b). Mandibular tusk shown in Fig. 2,–9; internal or secondary ramus of Gill 7 more elongate and conspicuous than in seventh instar, and the outer or primary ramus more complex.

? Tenth instar, (Fig. 2,–10, 10a, 10b, 10c and 10d). Mandibular tusk more developed, with a rather blunt apex, and a seta basally. Rostrum more concave on its anterior border than in seventh instar. Seventh gill (Fig. 2,–10b) with an elongation and basal enlargement of the internal or secondary ramus.

Eleventh instar, (Fig. 2,–11, 11a, 11b and 11c). Rostrum more emarginate and small setae appearing along anterior border; mandibular tusk longer than in previous instar and acutely instead of bluntly pointed; apically also it is much more heavily sclerotized than in the tenth instar; basally the tusk has two setae, one of which is longer and stronger than the other. Gill of seventh segment shows a change in the secondary ramus in the appearance of a bifid apex and the beginning of one medial filament.

? Thirteenth instar, (Fig. 2,–12, 12a, 12b, 12c and 12d). Rostrum has become more emarginate and six short setae are present around concavity in front of median ocellus. Mandibular tusk with much the same proportions as in previous instar, but longer, more heavily sclerotized and with four long setae proximally Setae on anterior border of femur more numerous than in tenth instar, and a row of seven short, thick setae has appeared in this position. Secondary or medial ramus of Gill 7 increased in length and with seven filaments, an addition of four to those started in the eleventh instar.

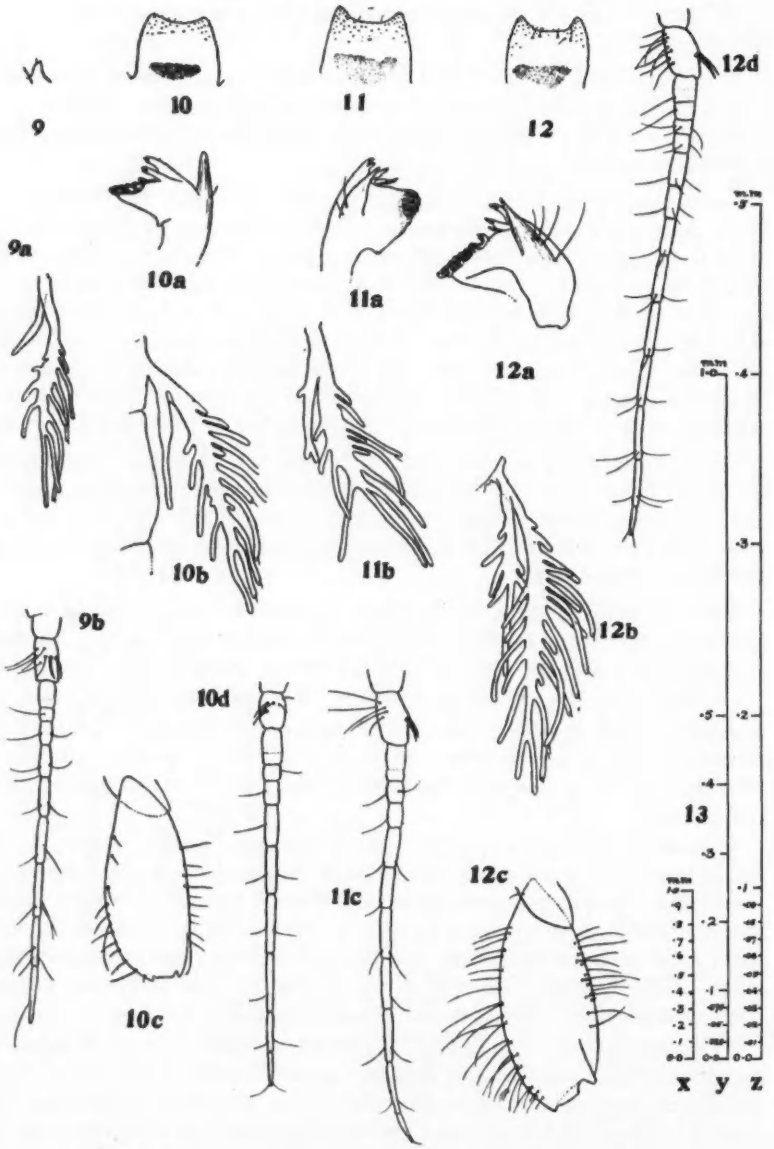

FIG. 2. *Ephemera simulans. 9-12d mag. Y. 9, Ninth instar, mandib. tusk; 9a, seventh gill; 9b, antenna. 10, Rostrum; 10a, mandible; 10b, seventh gill; 10c, hind femur; 10d, antenna. 11, Rostrum; 11a, mandible; 11b, seventh gill; 11c, antenna. 12, Rostrum; 12a, mandible; 12b, seventh gill; 12c, hind femur; 12d, antenna. 13, Scales of drawings with compound microscope using 10X ocular; X, using 16 mm. objective; Y, using 4 mm. objective; Z, using oil immersion*

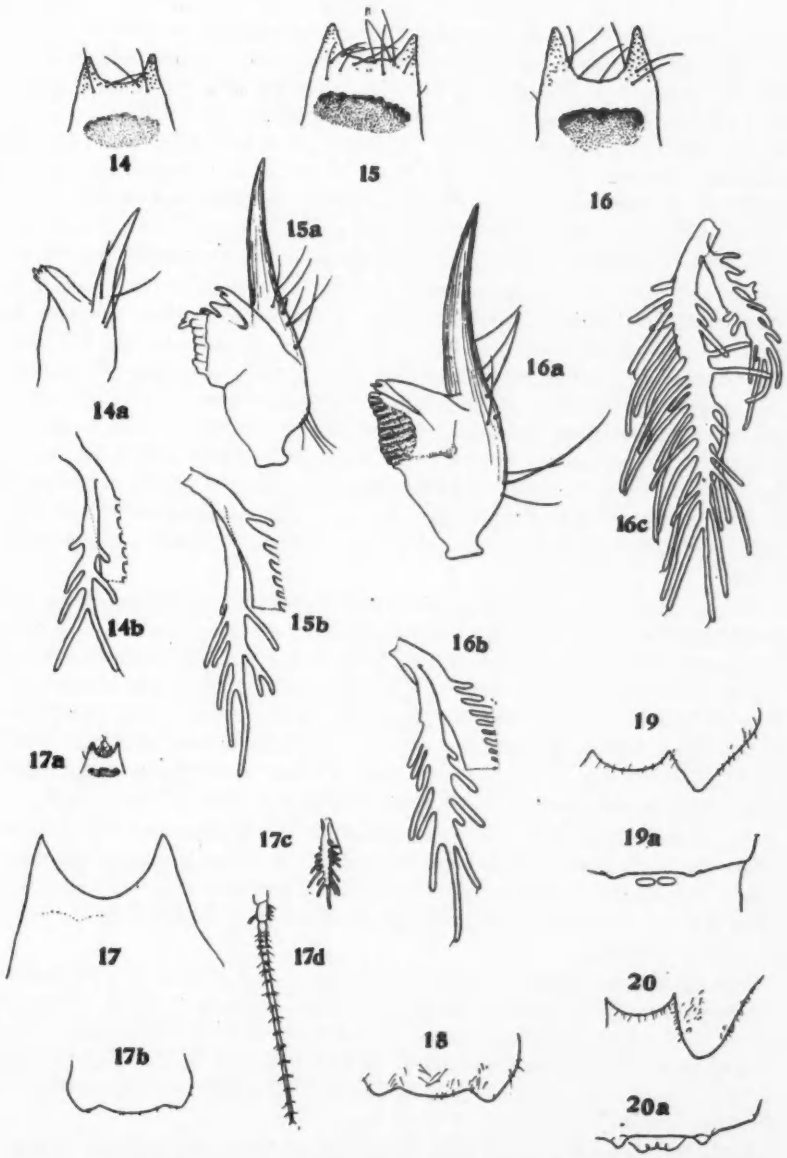

FIG. 3. *Ephemera simulans. 14-17 mag. Y. 17a-20a mag. X. 14, Rostrum; 14a, mandible; 14b, seventh gill. 15, Rostrum; 15a, mandible; 15b, seventh gill (ventral lobe entire); 16, Rostrum; 16a, mandible; 16b, seventh gill; 16c, seventh gill (entire). 17, Rostrum; 17a, rostrum enlarged; 17b, mesothoracic wing pads; 17c, seventh gill; 17d, antenna. 18, Mesothoracic wing pads. 19, Mesothoracic wing pads; 19a, male genitalia. 20, Mesothoracic wing pads; 20a, male genitalia.*

In Fig. 3,–14-16 are illustrated corresponding structures of three later instars which it will be seen at a glance cannot be consecutive instars. Fig. 3,–14, 14a, 14b, are taken from an individual which has 15 segments distinguishable in the antennal flagellum so that it is probably the 17th instar. Fig. 3,–15, 15a, 15b, are of a stage which is probably about the 18th instar, judging by the number of segments in the antennae. Similarly Fig. 3,–16, 16a, 16b, probably represent the 20th instar, since about 18 or 19 segments could be made out in the antennal flagellum. Adding to these one or two lost by fusion at the base the true number is calculated at about 20, and this, as we have seen in previous cases, gives approximately the number of the instar.

Through these stages the rostrum shows a deepening of the anterior concavity until its ultimate bifid character is developed. After the twentieth instar there is little change in its relative shape, merely an increase in size. The tusks of the mandibles also develop rapidly their final proportions during the stages immediately preceding the twentieth instar. The gills of the seventh segment show increasing complexity of structure which is easier to observe in the secondary ramus which alone is shown in full in Fig. 3,–14b, 15b, 16b. In Fig. 3,–16c is shown a complete seventh gill for a comparison of the secondary with the primary ramus. The wing pads have begun to form in this series probably in the stages immediately before the twentieth instar.

In Fig. 3,–17, 17a, 17b, 17c and 17d show structures of a later instar which is probably about the twenty-second, judging by the number of segments in the antennae, the increased size of the rostrum and the greater complexity of the secondary ramus of Gill 7. The wing pads in this instar reach back to a point in line with the posterior extremity of the mesonotum. The figures of this instar and subsequent ones are drawn at a lower magnification (Mag. X Fig. 2,–13), but Fig. 3,–17, of the rostrum is drawn to the same scale (Mag. Y, Fig. 2,–13) as that used in all previous figures as a basis of comparison.

Fig. 3,–18, shows the mesonotum of what is probably the next instar in which the wing pads project back beyond the posterior margin of the notum. There were about 23 segments discernible in the antennal flagellum. Obviously there is a gap between the instar represented in Fig. 3,–18 and that represented in Fig. 3,–19 and 19a.

During the last eight or nine instars the wing pads and male external genitalia are rapidly growing back so that the series is probably more complete, but even so it has some gaps; for instance there is apparently an instar missed between those represented in 19 and 19a, and in 20 and 20a, Fig. 3.

A system of nomenclature used by Dr. P. P. Calvert has been followed for the later instars.

? Nonult instar (Fig. 3,–19, 19a). In this instar the wing pads are triangular and measure about .29 mm. in length. The claspers in the male are present as mere rounded elevations on the posterior margin of the ninth sternum. The penes have made their appearance as elongate, slightly elevated tubercles on the intersegmental membrane behind the ninth sternum.

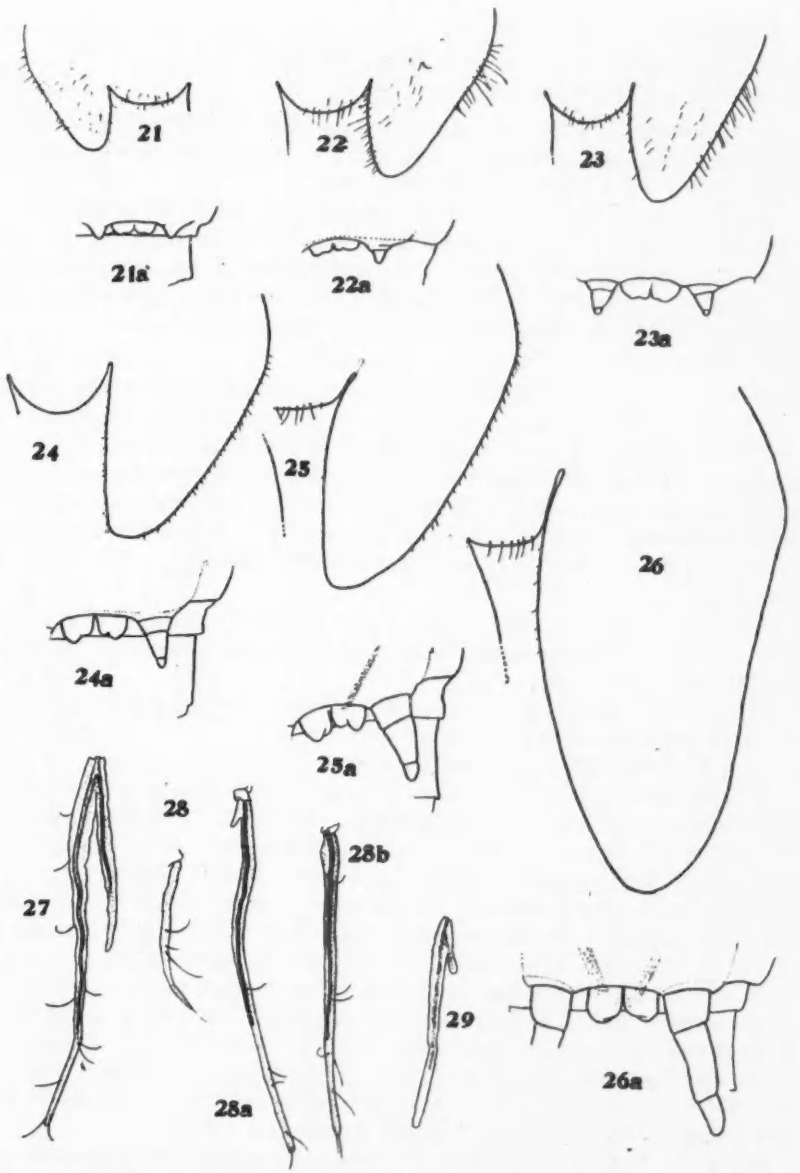

FIG. 4. *Ephemera simulans. 21-26a mag. X. 27-29 mag. Y. 21, Mesothoracic wing pads; 21a, male genitalia. 22, Mesothoracic wing pads; 22a, male genitalia. 23, Mesothoracic wing pads; 23a, male genitalia. 24, Mesothoracic wing pads; 24a, male genitalia. 25, Mesothoracic wing pads; 25a, male genitalia. 26, Mesothoracic wing pads; 26a, male genitalia. 27, Leptophlebia debilis; fifth gill of left side. 26, Leptophlebia debilis first gill; 28a, fourth gill; 28b, fifth or sixth gill. 29, Leptophlebia debilis; sixth gill of left side.*

Septult instar, (Fig. 3,–20, 20a). The penes (penes + parameres) now betray their double nature in the biloped condition of each.

Sextult instar, (Fig. 4,–21, 21a).

Quintult instar, (Fig. 4,–22, 22a). Wing pads are relatively longer than in previous instar; male claspers about .1 mm. in length, and of two segments, a short basal and a longer conical apical one.

Quartult instar, (Fig. 4,–23, 23a). Wing pads have increased to a length of .74 mm. which is not as great an increase as there was between the two previous instars; distance between wing pads about .5 mm. which is about the same as in the two former instars. The two distal segments of the claspers represent the single apical segment of the previous instar.

Tertiult instar (Fig. 4,–24, 24a).

Penult instar, (Fig. 4,–25, 25a). Wing pads have not increased very greatly in length, measuring now about 1.36 mm. Instead of being directed posteriorly as in previous instar, they converge noticeably at their apices. Male claspers have lengthened and lost some of their conical symmetry, but are still composed of three well defined segments. It is interesting to note that although there has been little increase in the length of the wing pad over that in the last instar, there has been a much greater increase in the length of the claspers.

Last instar, (Fig. 4,–26, 26a). Wing pads increased to a length of 2.6 mm. Towards the end of this instar the wing pads become first grayish and then black, prior to emergence.

The changes taking place in the caudal filaments from nymph to subimago and from subimago to adult are illustrated in Fig. 5.

A count of the single segments in a lateral caudal filament (Fig. 5,–30, 30a) of a female nymph of the last instar gives about 94 segments formed during the thirty or more instars. The subimago (Fig. 5,–32, 32a, 32b) has fewer (about 74) and the adult female (Fig, 5,–33) and male (Fig. 5,–34) have each about 72 segments. In spite of this great reduction in the number of segments the caudal filaments of the adult are much longer than those of the subimago, and those of the subimago are in turn much longer than those of the full-grown nymph. This great increase in length is produced by a great elongation and swelling of the segments, and not by any increase in their number. Indeed, there is actually a reduction in the number of segments in the change from nymphal to subimaginal state. The twelve distal segments of the nymphal caudal filament do not represent segments of the subimago or adult, but are dropped in the manner shown in Fig. 5,–31a, where the developing subimaginal filament could be made out within.

Fig. 5,–31, 31a, of the nymphal filament, show the developing subimaginal filament within and Fig. 5,–32, 32a, show the developing imaginal filament within the subimaginal. In Fig. 5,–30, 30a, the segments formed at each ecdysis are indicated, and in the other figures an attempt has been made to designate homologous segments similarly. A more detailed description of Fig. 5 will be given later in a discussion of segmentation.

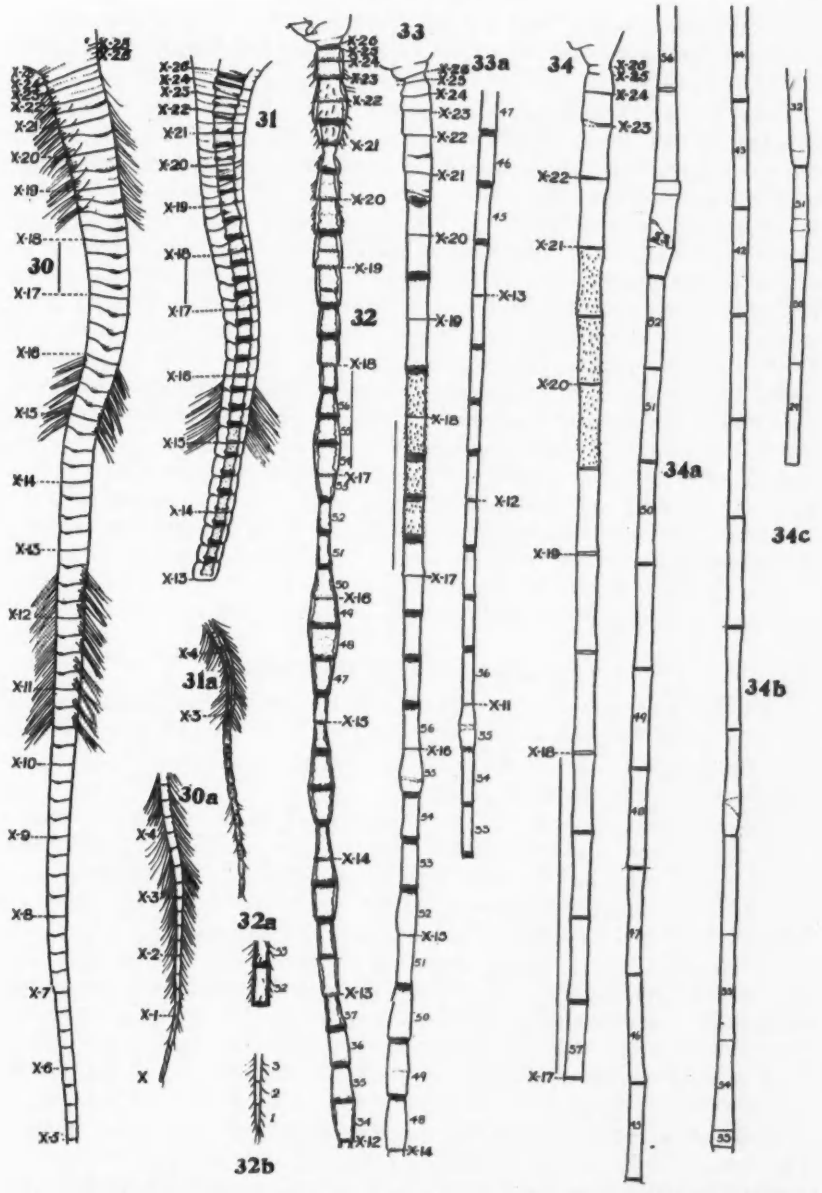

FIG. 5. *Ephemera simulans. All mag. X. 30 and 30a, Lateral caudal filament of female, last instar nymph. 31 and 31a, Lateral caudal filament of last instar female nymph, showing the subimago filament within. 32, 32a and 32b, Lateral caudal filament of female subimago showing lateral caudal filament of imago within. 33, 33a, Basal half of lateral caudal filament of adult female. 34, 34a, 34b and 34c, Basal portion of lateral caudal filament of adult male.*

In *Ephemera simulans* there is considerable metamorphosis during development, involving the antennae, caudal filaments, external genitalia, wing pads, eyes, mouth parts, gills and the number of setae and hairs.

The antennal segments are added one at each instar through most of the nymphal life and the caudal filaments add segments, in the following way,—one-half in the second instar, one in the third instar, one in the fourth instar, two in the fifth instar, three in the sixth instar, four in the seventh and in all subsequent nymphal instars. The twelve distal segments are dropped in the subimago. The mouth parts show considerable metamorphosis as shown by the appearance and rapid development of the mandibular tusks, beginning about the sixth and lasting to about the twentieth instar, after which the tusk increases at approximately the same rate as the body as a whole. The rostrum in its development keeps pace with the mandibular tusks. The gills make their appearance at the second instar as uniramous appendages, and increase in complexity and size during development. The internal or secondary ramus appears at the fifth or sixth instar, and develops rapidly, never, however, reaching the length nor the complexity of the primary ramus. The wing pads make their appearance at about the twelfth from the last instar, and grow out rapidly. The genitalia are not apparent as early as the wing pads, but are visible soon after, the claspers as rounded elevations on the posterior border of the ninth sternum, and the penes as rounded vesicles on the intersegmental membrane behind the ninth sternum.

Stenonema canadense Walker

The full-grown nymph of this species is well described by Clemens (1915).

The eggs are of a reddish color, and when shed into water adhere in a mass held together by a gelatinous material, and also by threads in skeins as described by Morgan (1913).

First instar, (Fig. 6,–1, 1a, 1b, 1c). Head as long as broad, posterior angles squarish; anterior border somewhat truncate; antenna with two basal segments and a flagellum in which one basal segment is differentiated and provided with an apical crown of spines, the remainder of the flagellum tapering and faintly marked off in pseudo- or sub-segments; compound eyes about the same size as lateral ocelli. The mouth parts were dissected out and are shown in Fig. 6,–1a, 1b, 1c (mandible, maxilla and labium); mandibles with cardinal teeth directed medially; molar surface not developed and there is an indication of a suture or joint across the mandible; maxilla and labium both unmodified and very different from the same structures in the full-grown nymph (Clemens, 1915).

In the thorax is the reddish, opaque mass of the yolk-containing enteron. Legs long and spindly, terminating in a long, scimitar-shaped, non-pectinate tarsal claw more than half the length of the tarsus; on each femur in the distal third a single posterior stout seta. Three subequal caudal filaments of which the telson or median is the longest; each filament composed of three well formed basal segments, each provided apically with a crown of spines,

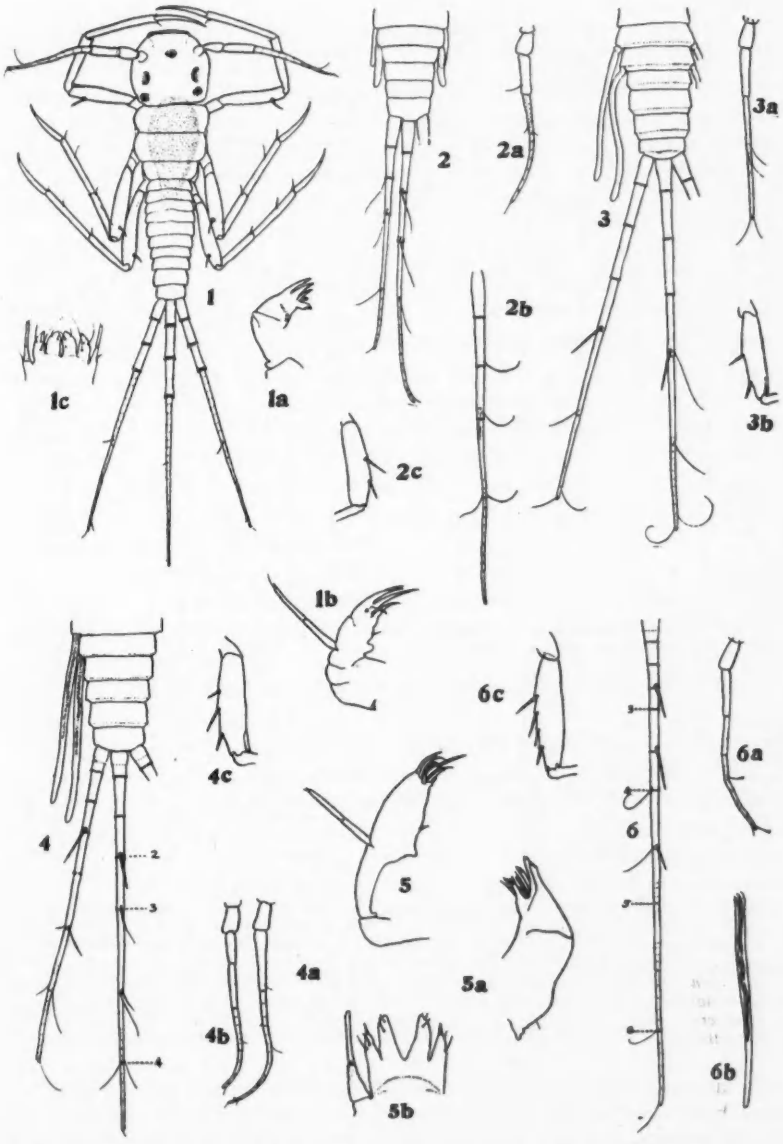

FIG. 6. *Stenonema canadense. 1a, 1b, 1c, 5, 5a, 5b, mag. Z; others mag. Y. 1, First instar; 1a, mandible; 1b, maxilla; 1c, labium. 2, Second instar; 2a, antenna; 2b, caudal filament, showing next instar within; 2c, femur. 3, Third instar, 3a, antenna; 3b, femur. 4, Fourth instar; 4a, antenna; 4b, antenna; 4c, femur. 5, Fifth instar, maxilla; 5a, mandible; 5b, labium. 6, Sixth instar, caudal filament; 6a, antenna; 6b, sixth gill; 6c, femur.*

TABLE II

DIAGNOSTIC CHARACTERS OF EARLY AND LATE INSTARS OF *Stenonema canadense*

Instar	Length in mm.			Segments in ant. flagellum, excluding terminal piece	Apparent no. of segments in antenna	No. of segs. in caudal filament, excluding terminal piece	Setae on posterior border of femur	Gills
	No. of individuals	Average	Lower and upper limits					
1	(20)	0.50	(0.45–0.55)	1		3	1	Absent
2	(28)	0.54	(0.47–0.59)	1½		4	2	On Segs. 5 and 6
3	(23)	0.62	(0.57–0.68)	2		5	2	On Segs. 5 and 6
4	(5)	0.69	(0.64–0.75)	3		7	3	On Segs. 5 and 6
5	(2)	0.72		3		9	3	On Segs. 5 and 6
6	(4)	0.81	(0.76–0.85)	4	3	11	4	On Segs. 5 and 6
7						14		On Segs. 5 and 6
8				6	5	18	7	On Segs. 1-7.
9						22		On Segs. 1-7.
10				6	5	26 (app. 24)		On Segs. 1-7.
11				7		30 (app. 28)	17	On Segs. 1-7.
12 to about 32								

	Length of mesothoracic wing pad of male, mm.	Distance between wing pads at base, mm.	Segments in male claspers	Length of male claspers
Octult	0.19			visible
Septult	0.32	0.37		visible
Sextult	0.36	0.48		visible
Quintult	0.50	0.50	1 segment	visible
Quartult	0.69	0.38	2 segments	visible
Tertiult	0.91	0.45	2 segments	0.16 mm.
Penult	1.16	0.58	2 (and indistinct basal)	0.26 mm.
Last	1.95	0.32	3 segments	0.30 mm.

The length of the nymph is taken from the front of the head to the end of the abdomen, excluding the length of the caudal filaments; the number of individuals and the upper and lower limits of the measurements for each instar appear in brackets. As with Ephemera the two basal antennal segments are omitted in the count; but in this case the terminal portion of the antennal flagellum has also been omitted. Similarly in the count of segments in the caudal filaments the terminal undifferentiated portion has been excluded from the count. The setae on the femur refer to the posterior series only. Owing to fusion of segments basally in the antennae and caudal filaments the true number cannot be made out. The apparent number in these cases is also listed.

and a tapering flagellum divided indistinctly into about twenty subsegments; about half way along this undifferentiated portion of the flagellum, but slightly proximad, a hair which probably marks the position of what will be the apex of the next segment to be formed. Gills absent, but in some individuals the postero-lateral angles of Segments 5 and 6 project more than the others owing, probably, to the formation of gills below the cuticle, which will appear at the next ecdysis.

Second instar, (Fig. 6,–2, 2a, 2b and 2c). The antennae have changed slightly; the small subsegments of the flagellum adjacent to the segment already formed in the first instar have shortened and fused, or in other words, another segment has been partially differentiated; antenna has lengthened by a corresponding amount. Gills on Segments 5 and 6, only, and measure from $1\frac{3}{4}$–$2\frac{1}{4}$ times the length of an abdominal segment. A fourth segment has been differentiated in the flagellum of each caudal filament and the filaments have been increased in length; distally in Segments 2, 3 and 4, a single long, fine hair. Fig. 6,–2b shows one caudal filament of this instar inside which the new filament of the next instar can be seen. A long stout seta is discernible on this filament, beneath the cuticle at the junction of Segments 3 and 4 of the old filament.

Third instar, (Fig. 6,–3, 3a and 3b). Antenna now with two segments basally in the flagellum, *i.e.*, the segment which was being differentiated in the last instar is now formed and has elongated, although there are still vestiges of the former subsegments of which it is composed. Gills present on Segments 5 and 6 only, but they have elongated so that they measure from five to six times the length of a typical abdominal segment. Apically in Segment 4 of the caudal filament a stout seta which was mentioned above in the description of the second instar.

Fourth instar, (Fig. 6,–4, 4a, 4b and 4c). Two conditions of the antennae were found. The commoner shown in Fig. 6,–4a had a very long basal segment in the flagellum, with a thickened base which is probably the fusion of two segments. The other condition Fig. 6,–4b, had a much shorter basal segment in the flagellum. Probably one segment has been added to those formed in the third instar.

Gills present on Segments 5 and 6 only, but longer than in the previous instar, measuring from seven to eight times the length of a typical abdominal segment; caudal filaments with seven segments differentiated basally, of which Segments 1 and 2 are rather intimately fused and shortened, so that the separation is not very easily made out in some individuals; distally in Segment 4 the stout seta mentioned above, and a somewhat weaker seta present apically in Segment 6. Two segments have been added this time in contrast to the single segment added in the previous instars.

Fifth instar, (Fig. 6,–5, 5a and 5b). Gills present on Segments 5 and 6 only, and each about seven times as long as a typical abdominal segment.

In Fig. 6,–5, 5a and 5b, the mouth parts are shown greatly enlarged. These show some change since the first instar. Terminal lobes of the labium longer; molar process of the mandibles more developed. At this instar the mouth parts are still very different from those of the last instar, as will be seen by referring to Clemens' figures of the full-grown nymph (Clemens, 1915). At the stage in which about thirteen segments are recognizable in the antennae, however, the mouth parts are very similar to those of the full-grown nymph, so that the change in these parts must be rather abrupt and occur early in the life of the nymph.

Sixth instar, (Fig. 6,–6, 6a, 6b and 6c). Caudal filaments now with eleven segments differentiated, so that two new segments have been added distal to those already formed in the previous instar.

In the sixth instar there is, as in the fifth, a stout seta apically on Segment 4 of the caudal filaments, and also one present apically on Segments 6 and 8; apically in Segment 11 a long, thin hair. In Fig. 6,–6, the portion of the filament which has been differentiated at each ecdysis is indicated.

In the seventh instar three segments have been added to those formed in the sixth instar. At every subsequent ecdysis four segments are added to the caudal filaments.

The change into the eighth instar seems to be a very critical stage in the life history of this species. This is the stage at which the gills make their appearance on Segments 1 to 4 and 7. Only one stage was taken which appears to be the eighth instar. The stages beyond the eighth were not reared from the egg, but collected in the stream.

?Eighth instar, (Fig. 7,–7, 7a, 7b). Gills now present on all segments from 1 to 7 inclusive; on Segments 1 to 4 as short, rounded appendages, on 5 and 6 linear as in the fifth instar, and on 7, linear gills in length equivalent to the eighth abdominal segment. The caudal filaments were broken off in this specimen.

Tenth instar, (Fig. 7,–8, 8a, 8b and 8c). Gills of Segments 1 and 2 shown enlarged in Fig. 7,–8a; Gill 7 (Fig. 7,–8c) about twice as long as in the preceding instar. In the caudal filaments there are about 24 segments apparent, with stout bristles present apically in Segments 6, 8, 10, 12, 16 and 20; seta of Segment 4 has been lost. The regions of the filament which were differentiated at each instar are enumerated in Fig. 7,–8.

Eleventh instar, (Fig. 7,–9, 9a. 9b). Gill 7 has increased in length so that it is nearly as long as Gills 5 and 6. The segments of the caudal filament formed at each instar are shown in the figure.

The femora are provided with about ten long setae and about seven shorter intercalary setae (Fig. 7,–9b).

?Twelfth instar, (Fig. 7,–10, 10a, 10b, 10c, 10d, 10e, 10f, 10g, and 10h). The number of this instar could not be determined with certainty. Anterior gills show an increase in length over the previous instar, and are shown under greater magnification; Gill 2 longer than others of the anterior series; Gills 5, 6 and 7 long and linear. Antennae with seven segments differentiated in the flagellum.

Fourteenth instar, (Fig. 7,–11 and 11a, 11b and 11c). At this stage the anterior gills have grown out to a fair size and are lamellate. As yet there is no appearance of the tuft of filaments at the bases of the gills. The posterior gills, 5 to 7 inclusive, are still long and linear, but their length has decreased in relation to that of the nymph.

The mesothorax is shown in Fig. 7,–11a. The postero-lateral angles, at which point the wing pads later develop, are quite evenly rounded.

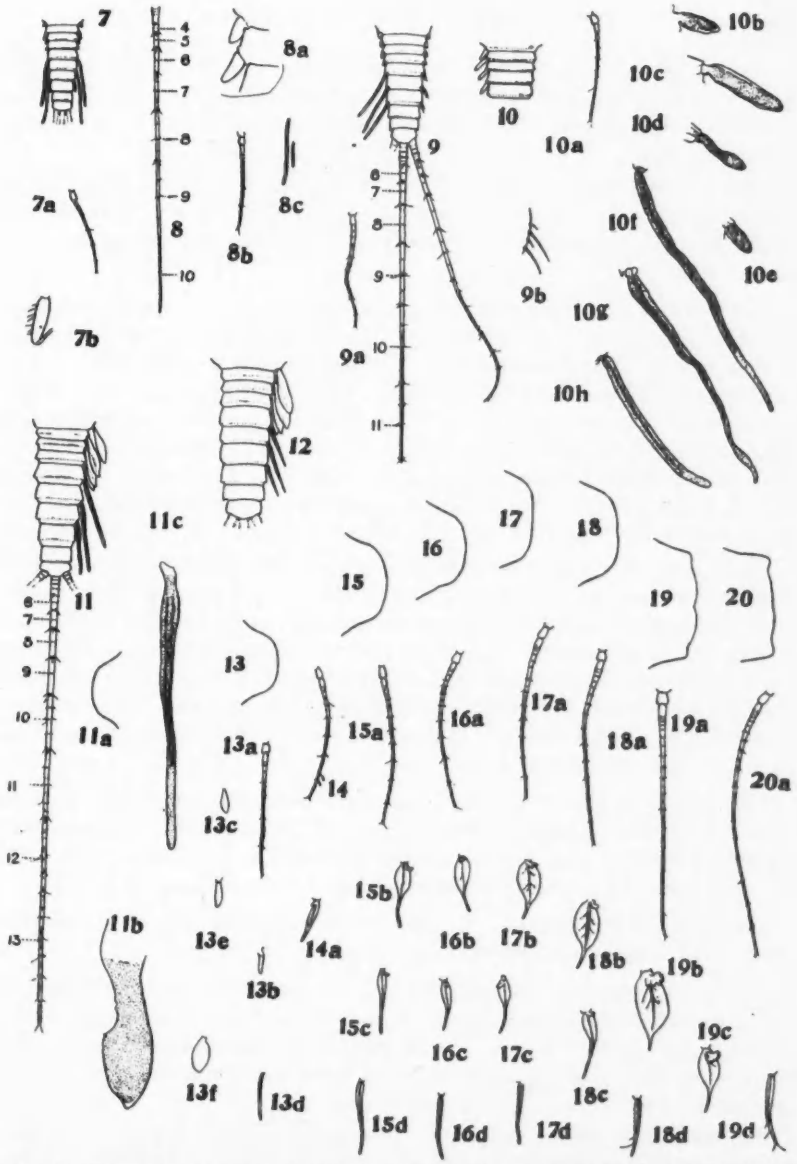

FIG. 7. *Stenonema canadense. 8a, 9b, 10b-10h, 11b, 11c, mag. Y; others mag. X. 7, ?Eighth instar, gills; 7a, antenna; 7b, femur. 8, Tenth instar, caudal filament; 8b, antenna; 8c, sixth and seventh gills; 8a, Gills 1 and 2. 9, Eleventh instar; 9a, antenna; 9b, part of femur. 10, Twelfth instar (anterior four gills); 10a, antenna; 10b–10h, Gills 1–7 under mag. Y. 11, Fourteenth instar; 11a, mesothorax; 11b, 11c, Gills 1 and 6. 12, Fifteenth instar. 13, Mesothorax; 13a, antenna; 13b, regenerating fifth gill; 13c, fourth gill; 13d, seventh gill; 13e, sixth gill; 13f, second gill. 14, Antenna; 14a, fifth gill. 15, Mesothorax; 15a, antenna; 15b, fifth gill; 15c, sixth gill; 15d, seventh gill 16, Mesothorax; 16a, antenna; 16b, fifth gill; 16c, sixth gill; 16d, seventh gill. 17, Mesothorax; 17a, antenna; 17b, fifth gill; 17c, sixth gill; 17d, seventh gill. 18, Mesothorax; 18a, antenna; 18b, fifth gill; 18c, sixth gill; 18d, seventh gill. 19, Mesothorax; 19a, antenna; 19b, fifth gill; 19c, sixth gill; 19d, seventh gill. 20, Mesothorax; 20a, antenna.*

?Fifteenth instar, Fig. 7,–12. Anterior gills larger and posterior ones have become smaller and more lanceolate; Gill 5 more affected by this process than 6, and in subsequent instars the former keeps ahead of the latter in its metamorphosis.

Beyond this point in the life history it was not possible to determine the number of the instar accurately from the material which was collected until nearer the end of nymphal life, when the last seven instars were fairly accurately determined.

Fig. 7,–13 to 20a, show the changes taking place in certain structures in an incomplete series of nymphs from a stage (13a) in which there were eight or nine segments differentiated in the antennae up to a stage (20a) in which nineteen segments were differentiated. Gradual change at each ecdysis is evident in the posterior border of the mesonotum, with the incidence of wing pad formation at about the middle of the series as a gradual prolongation of the postero-lateral angles of the notum. Gill 7 shows little change throughout the series, but Gills 5 and 7 show considerable change from the linear type found in Gills 5 to 7 in early life to the lamellate type of the anterior segments.

At the stage in which there are eight segments differentiated in the antennal flagellum, the ventral lobe of the fifth and sixth gills has made its appearance and during subsequent instars this lobe becomes more complex until finally it is a tuft of filaments. The basal part of these gills becomes more and more expanded and the terminal part shortens until finally Gills 5 and 6 from their very different appearance early in development come to resemble the anterior gills. Gill 7 does not change noticeably and never develops the tuft of filaments at the base.

Fig. 8,–21, 21a, 21b, 21c and 21d show parts of a later instar in which there are twenty segments differentiated in the antennal flagellum. The mesothoracic wing pads are more prominent than in previous instars. Gills 5, 6 and 7 are shown greatly enlarged in Fig. 8,–21b, 21c and 21d.

Fig. 8,–22, 22a, illustrate a later instar in which 21 or 22 segments are differentiated in the antennal flagellum.

Fig. 8,–23, 23a, 23b, 23c and 23d are of a later instar in which 23 or 24 segments are differentiated in the antennal flagellum. The mesothorax is shown in Fig. 8,–23. In Fig. 8,–23b and 23c are shown Gills 5 and 6 which are now much like the anterior gills and have lost the terminal filamentous portion.

Fig. 8,–24 and 24a are of a still later stage, in which about 25 segments are present in the antennal flagellum. The wing pads measure about .1 mm. in length. In this instar the developing external genitalia are not apparent, but in the next instar they can be made out so that it is possible from here on to separate male and female individuals on this character. With the wing pads and external genitalia developing through the next instars it was possible by an examination of a number of individuals to determine what are believed to be the last eight instars.

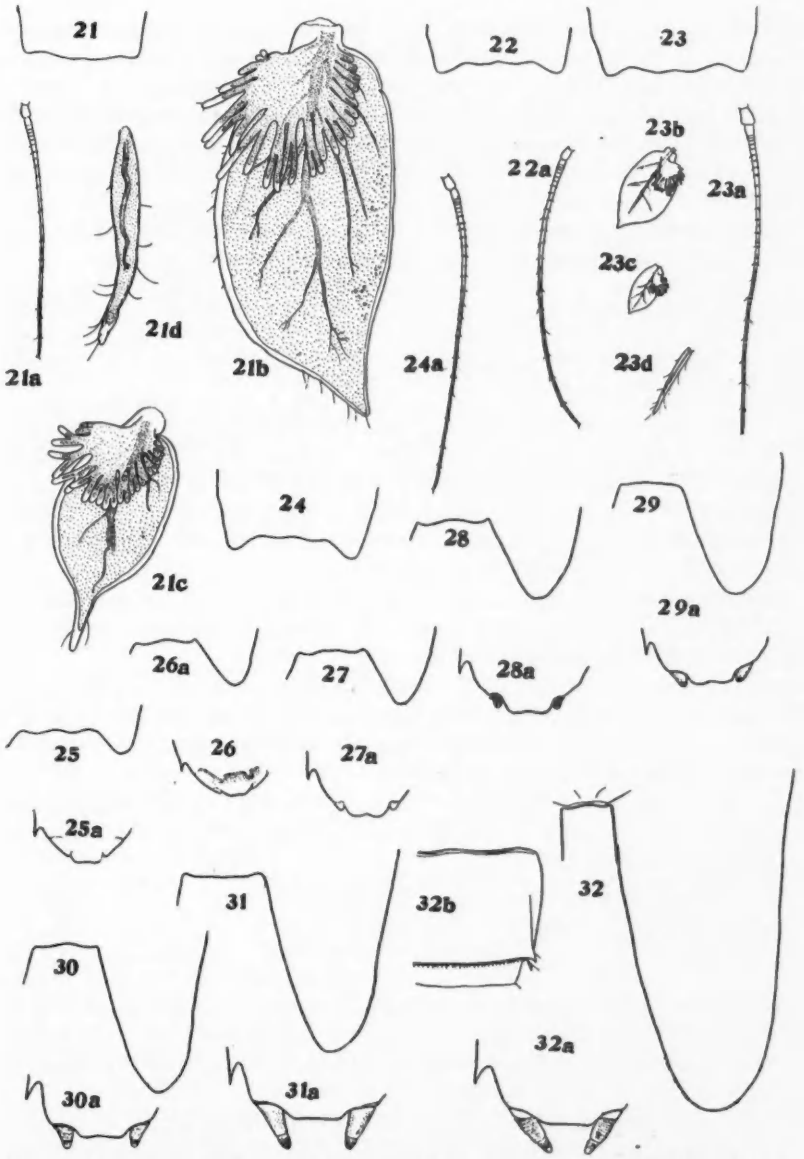

FIG. 8. *Stenonema canadense. 21b, c, d, mag. Y; others mag. X. 21, Mesothorax; 21a, antenna; 21b, fifth gill; 21c, sixth gill; 21d, seventh gill. 22, Mesothorax; 22a, antenna. 23, Mesothorax; 23a, antenna; 23b, fifth gill; 23c, sixth gill; 23d, seventh gill. 24, Mesothorax; 24a, antenna. 25, Mesothoracic wing pad; 25a, developing male genitalia. 26a, Mesothoracic wing pad; 26, male genitalia. 27, Mesothoracic wing pad; 27a, male genitalia. 28, Mesothoracic wing pad; 28a, male genitalia. 29, Mesothoracic wing pad; 29a, male genitalia. 30, Mesothoracic wing pad; 30a, male genitalia; 31, Mesothoracic wing pad; 31a, male genitalia. 32, Mesothoracic wing pad; 32a, male genitalia; 32b, seventh abdominal tergum.*

Octult instar, (Fig. 8,–25, 25a). The developing male claspers may be distinguished at this stage as small indentations on the postero-lateral margins of the sternum. There is a suture separating the posterior portion of the sternum from the anterior sternite proper, which suggests that this posterior part of the sternum may be the fused appendages of this segment, part of which will develop into the claspers and the medial part be associated with the penes.

Septult instar, (Fig. 8,–26, 26a). Male claspers have not increased much in size and are rather difficult to distinguish as in the previous instar.

Sextult instar (Fig. 8,–27, 27a). Male claspers not very distinct, but visible as rounded projections on the lateral borders of the sternum; their bases visible; they extend less than half way from their bases to the posterior border of the ninth sternum.

Quintult instar, (Fig. 8,–28, 28a). Male claspers composed of one segment only, darkly pigmented with a pale tip, extending only slightly more than half way from their bases to posterior border of ninth sternum.

Quartult instar, (Fig. 8,–29, 29a). Male claspers of two segments; small distal segment pigmented to tip; claspers reach the level of posterior border of ninth sternum or project very slightly beyond it.

Tertiult instar, (Fig. 8,–30, 30a). Apical segment of clasper pigmented in proximal half and unpigmented distally; basal segment pigmented, the pigment being distributed in annuli.

Penult instar, (Fig. 8,–31, 31a). Male claspers about .26 mm. in length, composed of two distinct segments and an indistinctly marked off basal segment; middle segment of clasper long and smooth; apical segment short and darkly pigmented distally to the apex.

Last instar, (Fig. 8,–32, 32a, 32b). In Fig. 8,–32b is shown the tergum of the seventh abdominal segment. The females are larger as a rule, having a wing pad length of about 2.2 mm.

The changes taking place in the caudal filaments from nymph to subimago and from subimago to imago are illustrated in Fig. 9,–33 to 36. In Fig. 9,–33, 33a, 33b, of the caudal filament of penultimate nymphal instar, there are about 122 segments, formed as indicated in about 40 instars. In the subimago and imago filament there are decidedly fewer segments, about 70 in the imago. This reduction may come about as in *Ephemera*, by the dropping of distal segments and probably also by the loss of a few basal segments by fusion and obliteration of the sutures.

There is considerable metamorphosis of structure during nymphal development in *Stenonema canadense*. Some structures, such as antennae and caudal filaments, develop continuously with the growth of the nymph, maintaining their length with little increase in relation to the size of the nymph. These structures, particularly the caudal filaments, are the most sensitive indicators of change, and record every ecdysis as far as can be made out. Other structures, such as the mouth parts, wing pads and external genitalia,

develop during a particular period of the nymphal life and during this time change greatly relative to the changes in the nymph as a whole.

The mouth parts change radically during the early instars, but by about the twelfth have a form similar to that of the full-grown nymph. The gills show definite metamorphosis apart from an increase in size. The gills of Segments 5 and 6 appear at the second instar and those of Segments 1 to 4 and 7 do not make their appearance until about the eighth instar. Gills 1 to 4 grow directly into flat lamellae, but Gill 7 becomes long and linear and similar to Gills 5 and 6. The latter pair gradually metamorphose until they are similar to Gills 1 to 4, but Gill 7 remains as a single lanceolate appendage known as the vestigial gill. Gills 1 to 6 develop a tuft of filaments ventrally near the base.

The wing pads make their appearance about the fifteenth from the last instar, and grow back at first rather slowly, then by gradually accelerated growth. The external male genitalia are first distinguishable in about the eighth from the last instar. The claspers in this instar are slightly rounded projections from the ninth sternum and the anlage of the penes lies between them. The caudal filaments at hatching have three segments differentiated basally and a terminal undifferentiated termina part to the flagellum. In the second instar there is one further segment differentiated in the caudal filaments, making four in addition to the terminal piece, which, however, is only about half as long as in the first instar. Apparently there has been very little growth during the first instar. In the third instar there are five segments differentiated, and the fourth bears a stout seta apically. In the fourth instar there are seven segments differentiated, showing that two segments are being added at each instar instead of one as in the earlier instars. In subsequent instars two segments are added up to the seventh, when three segments are added. In the eighth instar and in all subsequent instars, four segments apparently are added at each ecdysis except in the subimaginal and imaginal instars. The individual new segments added at each instar are of approximately equal length and volume in the early stages, so that change in the rate of growth is indicated by the manner in which the segments are added, the supplemented segments increasing in number in the ratio of 1 : 1 : 2 : 2 : 2 : 3 : 4 : 4, from the first to the ninth instars.

Curiously it is at the eighth instar that the gills make their appearance on Segments 1 to 4 and 7, a fact which may be more than coincidental since the increase in rate of growth probably implies a greater oxygen requirement. Also, as mentioned above, this stage is a very critical one in the life of the nymph as indicated by failure to rear them beyond this stage in conditions under which they incubated well and passed through the first stages successfully.

In Fig. 9,–33 a complete lateral caudal filament of a female individual of the next to last nymphal instar is shown. The portions of the filament laid down at each instar are indicated and a count of these regions beginning at the apex reveals that there are 39 instars recorded which, with the last,

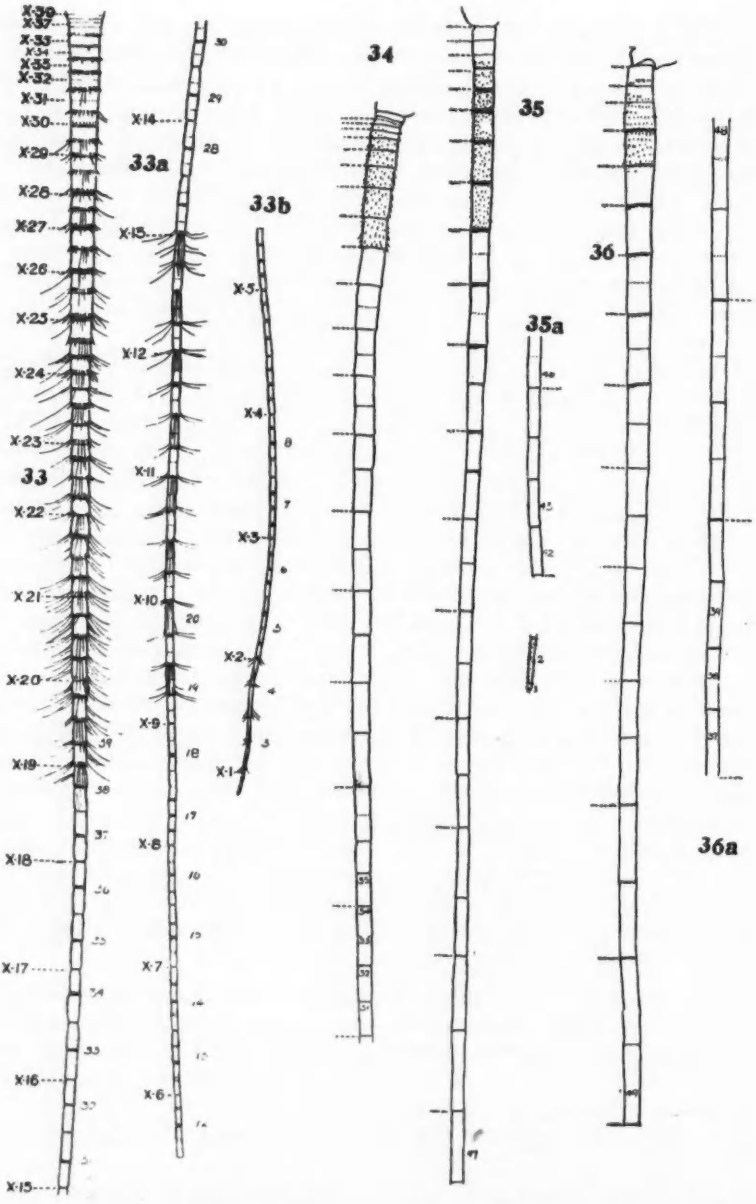

FIG. 9. *Stenonema canadense. All mag. X. 33, 33a, 33b, Complete lateral caudal filament (cercus) of a female nymph in the penultimate instar. 34, Lateral caudal filament of subimaginal female. 35, 35a, Lateral filament of adult female; 36, 36a, Lateral filament of adult male.*

make 40. Unfortunately, this is not the full number of instars represented during the nymphal period. Fusion of segments at the base and their almost entire obliteration results in a smaller number than was actually differentiated during development. There are probably between 40 and 45 instars in this species. Of these, 35 have been illustrated showing how they differ morphologically, and obvious gaps in the sequence noted.

Ephemerella subvaria McDunnough

An account of the full-grown nymph of this recently described species is given by McDunnough (1931). This was a common species in the streams studied by the author and females were frequently taken ovipositing. The eggs are extruded in a spherical mass of a brownish yellow color, which is dropped in flight into the water of the rapids.

The eggs are ovoid in shape, finely reticulate, and measure about .2 by .15 mm. They are stuck end to end by a mass of very elastic gelatinous material so that when the string of eggs is stretched this material pulls out into a thin elastic thread. Sometimes the strings branch and anastamose, three eggs instead of two being held together by one mass of the elastic material.

First instar, Fig. 12,–8. Three individuals measured on an average .495 mm. in length, exclusive of the caudal filaments. The head is somewhat triangular with the apex directed more ventrally than in *S. canadense;* antennae with two distinct basal segments, a very short proximal and a longer distal one, and a flagellum in which three segments are distinguishable including the apical portion; in front of each compound eye a curved hair.

Each of the femora bears distally a curved hair or fine seta; tarsal claws pointed and with two rows of pectinations converging on the large apical claw.

Caudal filaments relatively short, equalling about one third the length of the body; three well developed basal segments and an apical undifferentiated part which shows in some cases an indication of weak joints; median caudal filament slightly longer than the lateral ones; gills absent.

Isonychia bicolor Walker

The full-grown nymph of this species is described by Needham (1905) under the name *Chirotenetes albomanicatus* Needh. which is placed as a synonym by McDunnough.

The eggs, (Fig. 12,–2), are spherical in shape with a diameter of .25 mm. They are extruded from the female in the form of a spherical mass of an olive green color, in which form they are dropped on the surface of the water during flight, and quickly disperse.

First instar, (Fig. 12,–1). The average length of six individuals was .71 mm. with a range of from .67–.75 mm. Gills have not made their appearance

and the yolk is still present as the opaque body shown in stipple. The compound eyes are less prominent than the lateral ocelli and are situated directly behind them.

Antennae with two basal segments and a tapering, flexible flagellum in which one proximal segment has been differentiated; tarsal claws pectinate; femora with only one rather stout distal seta on the posterior border and a somewhat weaker dorsal one; three caudal filaments each consisting of three well developed proximal segments, each of which bears a conspicuous crown of spines and a terminal undifferentiated part bearing a few hairs.

Second instar, (Fig. 12,–3, 3a, 3b). Gills have not appeared; antennae with no increase in number of segments; still but one segment differentiated proximally in the flagellum; the terminal portion of the flagellum is not, however, tapered as in the first instar, but more cylindrical with a slight constriction in the middle; setae on posterior border of femora increased to two on fore femur and three on hind femur, on which they are well developed, the middle one being the longest; caudal filaments show a definite change in the addition of one segment, making four well differentiated segments proximally; this change may easily be overlooked, however, since the crown of spines on the proximal segment has been so reduced that it is visible only with the closest observation. The new distal segment (Segment 4), has been added from the undifferentiated distal part of the filament of the first instar; basal Segments 1 and 2 intimately fused together, the crown on Segment 1 being almost obliterated. In the next instar it is very probable that the separation between 1 and 2 will not be apparent, the fusion being complete, and so on through the life of the nymph, segments are formed apically and proximal ones lose their identity in the process of fusing to form a strong rigid base for the filament.

One individual in this stage had a portion of the exuvia of the first instar attached, showing plainly the single spine distally in the third femur, and also the three-segmented condition of the bases of the caudal filaments. The fusion of Segments 1 and 2, which makes it impossible to distinguish them in some individuals and very difficult in others, probably led to Lubbock's conclusion that growth takes place by an elongation of the basal segment and its ultimate division into two. In describing the second instar of *Cloeon dimidiatum*, Lubbock, (1864) writes: "The two tails have increased to a length of 25/200, and consist of twenty segments." (It should be mentioned that this large number of segments is due to the fact that Lubbock includes the small subsegments of the flagellum in his count.) "Here again as in the antennae almost the whole change has taken place in one segment, which however, is in the present case the basal one. The remainder are almost exactly as they were before. As already mentioned the basal segment was in the first stage 5/800 in length; in the present it has divided into two segments which, taken together, are 9/800 and we see therefore that almost the whole increase of length is in this one part."

In another section of the present paper the addition of segments has been discussed and it is evident that segments are added from the distal end to those already present, and not by a division of segments which have been differentiated. The basal segments are not becoming shorter by being subdivided, but by thickening and fusing together.

Fig. 12,–4, 4a, 4b show a later instar whose number was not determined owing to the absence of antennae and caudal filaments which had been broken off. From its size (1.20 mm.) and the number of setae on the femur, it is probably about the fourth. At this stage the gills are making their appearance as rounded projections from the postero-lateral angles of the segments. They are slightly longer on Segments 4, 5 and 6, but probably appear simultaneously on all segments as was the case with *Ephemera simulans*. The claws are pectinate.

These early stages were reared from the egg in water from the stream standing in an open jar. Eggs from several females were set to incubate on July 6, 1931. On July 24 some had hatched although none were out on July 21. Four first instar individuals were taken in a sample from the jar on July 24. On July 28 a sample was taken which contained one individual in the first instar and twenty in the second instar. On August 11 one individual was removed from the jar and this one was in about the fourth instar. No others were seen on this date. On August 15 one first instar nymph was taken. Development in this species is apparently very rapid. The temperature of the water in the jar was of course higher than is normal for hatching of this species.

Epeorus humeralis Morgan

The full-grown nymph is described by Dr. Anna Morgan (1911).

The eggs (Fig. 10,–1a) which are of a reddish color, are extruded from the female in two ribbon-like masses but quickly disperse on touching the water. The length is about .20 mm. and the width .12 mm. They are not provided with special organs of attachment.

First instar, (Fig. 10,–1). The average length of six nymphs was .40 mm., the limits being about .33 to .42 mm. The head broader than long and convex in front; the compound eyes (the pigmented portion of them at least) smaller than the lateral ocelli; antennae with two basal segments and a flagellum in which one segment is differentiated basally; portion of flagellum distal to this segment divided into small faintly demarcated subsegments as mentioned above in the description of *S. canadense*. The opaquely reddish mass of the enteron is visible through the integument of the thorax. This is shown as a stippled area in the figure.

Abdomen without gills; lateral caudal filaments (cerci) with three segments differentiated at the base, each with a crown of spines apically; remaining portion of caudal filament tapered and divided into small subsegments; median caudal filament with one basal segment and a distal three-segmented flagellum.

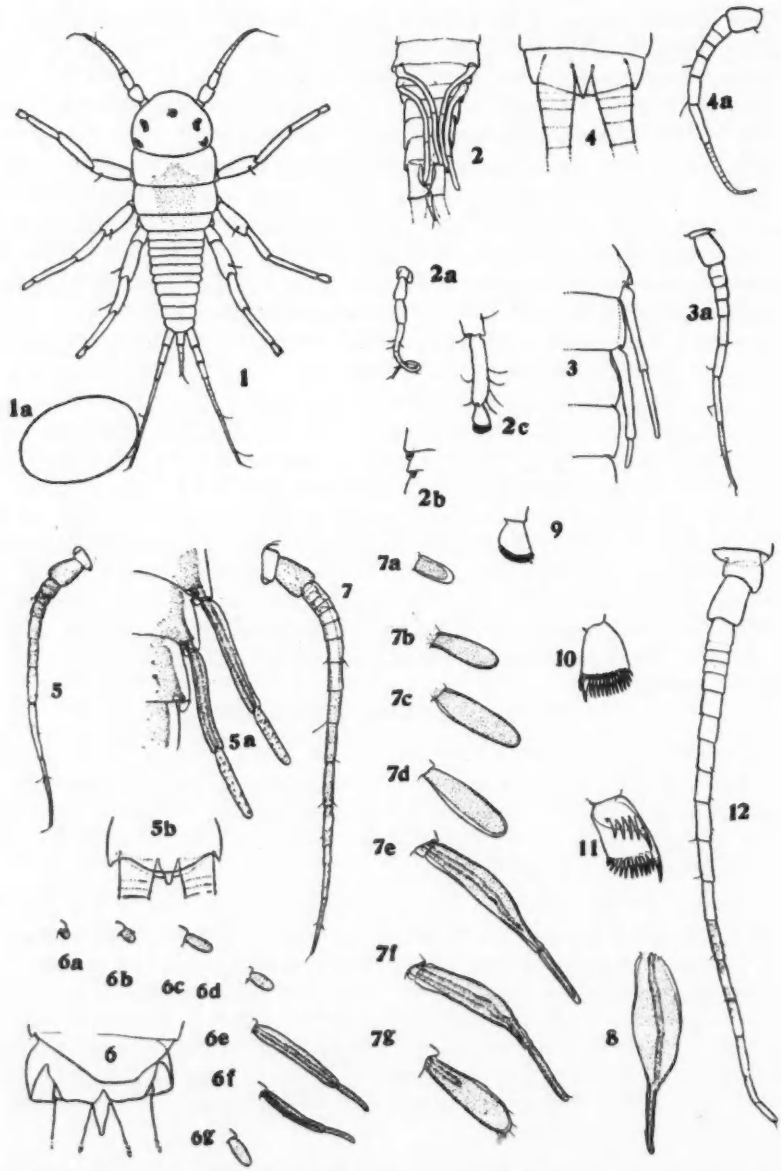

FIG. 10. *Epeorus humeralis. All mag. Y. 1, First instar; 1a, egg. 2, Ventral view of later instar; 2a, antenna; 2b, dorsal view of Gills 5 and 6; 2c, tarsus and tarsal claw. 3, Later instar, Gills 5 and 6; 3a, antenna. 4, Posterior end of earlier instar showing reduced median caudal filament; 4a, antenna. 5, Antenna; 5a, Gills 5 and 6; 5b, posterior end of abdomen. 6, Post end of abdomen, ventral view; 6a–g, Gills 1–7. 7, Antenna; 7a–g, Gills 1–7. 8, Gill 6. 9–11, Tarsal claws of 3 instars last showing new type of claw within. 12, Antenna.*

A later instar (Fig. 10,–2, 2a, 2b and 2c) shows two well developed segments in the antennal flagellum and measures .73 mm. Gills are present on Segments 5 and 6 only, long and filamentous, directed ventrally, and divided into two segments, a long basal and a shorter distal one. The fact that the gills are directed ventrally makes them very inconspicuous, and it was some time before they were noticed.

Lateral caudal filaments thick and bulbous at the base, narrowing somewhat suddenly into the main filament which had dropped off in most individuals; median caudal filament present as a short three-segmented appendage; tarsal claws are particularly interesting, consisting of a series of about 15 curved hooks of nearly equal size. One of these at one end of the series is, however, stouter. In development it is this stouter claw which will become the tarsal claw proper and those of the others which remain will become the pectinations along one side of the main claw.

In a later instar (Fig. 10,–4, 4a) in which there are six segments in the antennal flagellum, the terminal segments of the median caudal filament are dropped, leaving a short conical projection in its place. A later stage (Fig. 10,–3, 3a) is shown in which the antennal flagellum has about seven segments. The gills are present on Segments 5 and 6, but are now directed laterad rather than ventrad.

In the stage in which eight segments are formed in the antenna (Fig. 10,–5, 5a and 5b), the gills are present on 5 and 6, as two-segmented structures, and the gills of some of the other segments have appeared as mere rounded elevations on one or two of the other segments. The relative sizes of the gills (Fig. 10,–6, 6a–g) are shown on all segments in a nymph measuring 1.9 mm. The gills of 5 and 6 are still two-segmented. The gills are also present now on Segments 1, 2, 3, 4 and 7 as short, rounded appendages. The gills are shown (Fig. 10,–7, 7a–g) at the stage in which the antennae have 12 segments differentiated. Those on Segments 1-4 and 7 are becoming lamellate but are still rather thick and the tracheae are not apparent. The tracheae are showing in the basal segments of Gills 5 and 6. The latter pair are broadening at the base. Length of nymph is 2.33 mm. Gill 5 is shown (Fig. 10,–8) in a later stage in which the nymph measured 3.4 mm. Gills 5 and 6 are now very similar to the other gills, except that they are formed of two segments, the distal one of which is not becoming lamellate. The antennal flagellum at this stage is composed of 16 well developed segments.

The terminal segment of Gills 5 and 6 is dropped after the stage in which the antennae contain 22 well developed segments in the flagellum and is absent from the later instars.

Fig. 11,–13, 13a–d illustrate some of the characters of this stage which measured 4.13 mm. Gill 6 still has a vestige of the terminal part which is dropped at the next shedding. The wing pads have been developing through several ecdyses. The tarsal claw has changed, the number of hooks or claws having been reduced from about fifteen in the stage illustrated on Fig. 10,–10, to five of which one is much longer than the others and more curved. The smaller ones form now the pectinations of the larger claw.

Fig. 11,–14 and 14a–e show some structures of the last instar. Fig. 11,–14c is of the tarsal claw of the third leg, which now consists of one great, curved claw bearing four smaller straight ones as pectinations. Gills 1, 5 and 7 are shown in ventral view. Of particular interest is the chitinous thickening around the outer margin of the gills armed with numerous thickly set short stiff spines directed downward and backward, which have been described by Morgan (1913). When the nymph is facing upstream into the current, as they usually do, these spines are in contact with the stone on which it is resting, thus firmly anchoring the nymph to the rock. It should be noticed that the limpet-like adaptation is not as perfectly developed in *Epeorus* as in *Iron pleuralis*, since *Epeorus* lacks the long anterior process which is present on Gill 1, of *Iron*, and also the fold in the seventh gill of the latter, which would allow the gills of the seventh segment to fit more closely together. The appearance of the genitalia of female and male which have been developing during the later instars is shown in Fig. 11,–14d and 14e respectively.

Curiously enough the abrupt transformation of the tarsal claws illustrated in Fig. 10,–11, coincides with the point in development at which the gills have grown out far enough to come in contact with the stone on which the nymph is resting, thus aiding in anchoring it. It also coincides roughly with the time in the life of the nymphs at which there is a marked tendency to drop out of the rapids into the somewhat quieter water below. This was determined in an ecological study of this species, which is presented in another paper, (Ide, 1935).

The nymphs of this species occur commonly along with those of *Iron pleuralis*, the next species, but in the younger stages can be readily distinguished from the latter in the field by the presence of a light yellow area on the anterior part of the dorsum of the abdomen, which is contrasted with the dark greenish color of the rest of the nymph. As a young nymph *Iron pleuralis* is uniformly light brown.

Iron pleuralis Banks

The full-grown nymph of this species has already been described, with some notes on its habits (Ide, 1930).

This species follows *Epeorus humeralis* very closely in its post-embryonic development. The earliest stage collected in the stream is one (Fig. 11,–15, 15a and 15b), in which the antenna has but three well formed segments in the flagellum, and which measures about 1.10 mm. in length. The lateral caudal filaments have 19 segments and the median filament is vestigial having but two segments present. Gills are present on Segments 5 and 6 only, where they are very long and filamentous, and composed of two segments, a distal short and a basal long one.

At the stage (Fig. 11,–16, 16a–f) in which there are six segments in the antennal flagellum, the gills on Segments 1, 2, 3, 4 and 7 have appeared as minute bud-like processes. The gills on Segments 5 and 6 are composed of two segments, but have become somewhat stouter. The median caudal filament

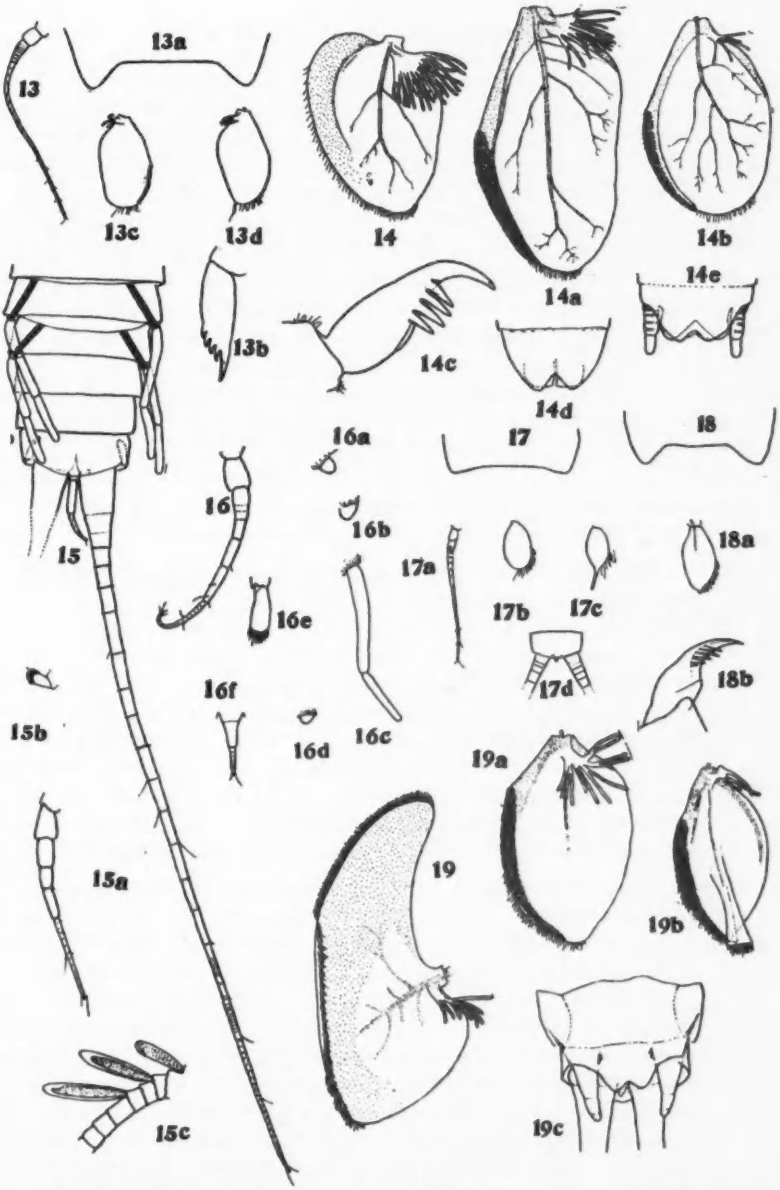

FIG. 11. *Epeorus humeralis. 13b, 14c, mag. Y. others mag. X. 13, Antenna; 13a, mesothoracic wing pads; 13b, tarsal claw; 13c, fourth gill of right side; 13d, sixth gill of right side. 14, Last nymphal instar Gill 1, ventral view; 14a, Gill 5; ventral view; 14b, Gill 7, ventral view; 14c, tarsal claw; 14d, posterior end of female, ventral view; 14e, male genitalia.*

Iron pleuralis. 15–16f, and 18b, mag. Y; 17–19c mag. X. 15, Nymph about seventh instar; 15a, antenna; 15b, tarsal claw; 15c, parasitic fungus protruding from the anus. 16, Antenna; 16a, b, c, d, Gills 1, 2, 5, 7; 16e, tarsal claw; 16f, median caudal filament. 17, Mesothorax; 17a, antenna; 17b, Gill 3; 17c, Gill 6; 17d, posterior end of abdomen, showing reduced median caudal filament. 18, Mesothorax; 18a, Gill 6; 18b, tarsal claw. 19, Last nymphal instar, Gill 1; 19a, Gill 5; 19b, Gill 7; 19c, male genitalia, ventral view.

is similar to the last described stage except that Segment 2 has become relatively shorter and more conical. The tarsal claws of *Iron pleuralis* differ from those of *Epeorus* in that the most strongly developed claw is near the middle of the series of claws rather than at one end, as was the case in the latter. Length in this stage is about 1.48 mm.

A later stage is illustrated (Fig. 11,–17, 17a–d) in which the specimen is 2.90 mm. long and the antenna contains 14 well developed segments in the flagellum, showing that the nymph has passed through several moults since the last described instar. The gills are now flat lamellae as shown in Fig. 11,–17b, 17c for gills of Segments 3 and 6 respectively. The terminal segment is present on Gills 5 and 6 as a narrow, cylindrical process. The distal segments of the median caudal filament have now been dropped (Fig. 11,–17d). About two instars later, when the antennae have about seventeen segments, the terminal segments of Gills 5 and 6 are dropped (Fig. 11,–18, 18a, 18b). The sixth gill is shown in Fig. 11,–18a, with a slight tubercle at its apex, the remnant of the former distal segment. At this stage the gills are all rather similar, although they have originated at different times in development.

From now on the metamorphosis is chiefly in the growing wing pads, the external genitalia, the antennae and caudal setae. In Fig. 11,–19, 19a–c, the detail of Gills 1, 5 and 7 of the last instar is given. The most notable feature is the anterior extension of Gill 1 which passes beneath the thorax ventrally, where it nearly meets the corresponding gill of the opposite side. In the gill of the seventh segment there is a fold medially directed in such a way that the gills of both sides nearly meet below the abdomen. All the gills, by this arrangement, form a sucking disc, the water pressure keeping the gills firmly pressed against the rock. Along the lateral borders, where the gills meet the rock, are series of short spines directed backwards, which form an efficient anchor, as Morgan has pointed out for *Iron fragilis* (1913).

In this species as in *Epeorus* there has been a considerable metamorphosis in gills and tarsal claws. In the younger stages the gills are not developed as organs of retention and so the whole load comes on the tarsal claws, which are numerous, small and arranged in a V-shaped line at right angles to the force so that they provide a maximum grip. The nymph is so small that it is only the minute irregularities of the surface of stones which are of use to it for retention, and in order to grip these irregularities there must be many, closely set, tarsal claws. As the nymph grows in size the gills become important as gripping organs and the claws change, until ultimately there is one large tarsal claw with pectinations along the inner border. These pectinations are derived from some of the smaller tarsal claws present in the early stages of development. The claws of the larger nymphs are probably used more for crawling than for clinging to the surface of the stone, and the irregularities against which they pry are not of the order which are useful earlier in the life history but much larger ones which are naturally much farther apart. A series of small tarsal claws in the form of a rake probably would not be as efficient now as the single pectinate claw. This is an interesting example

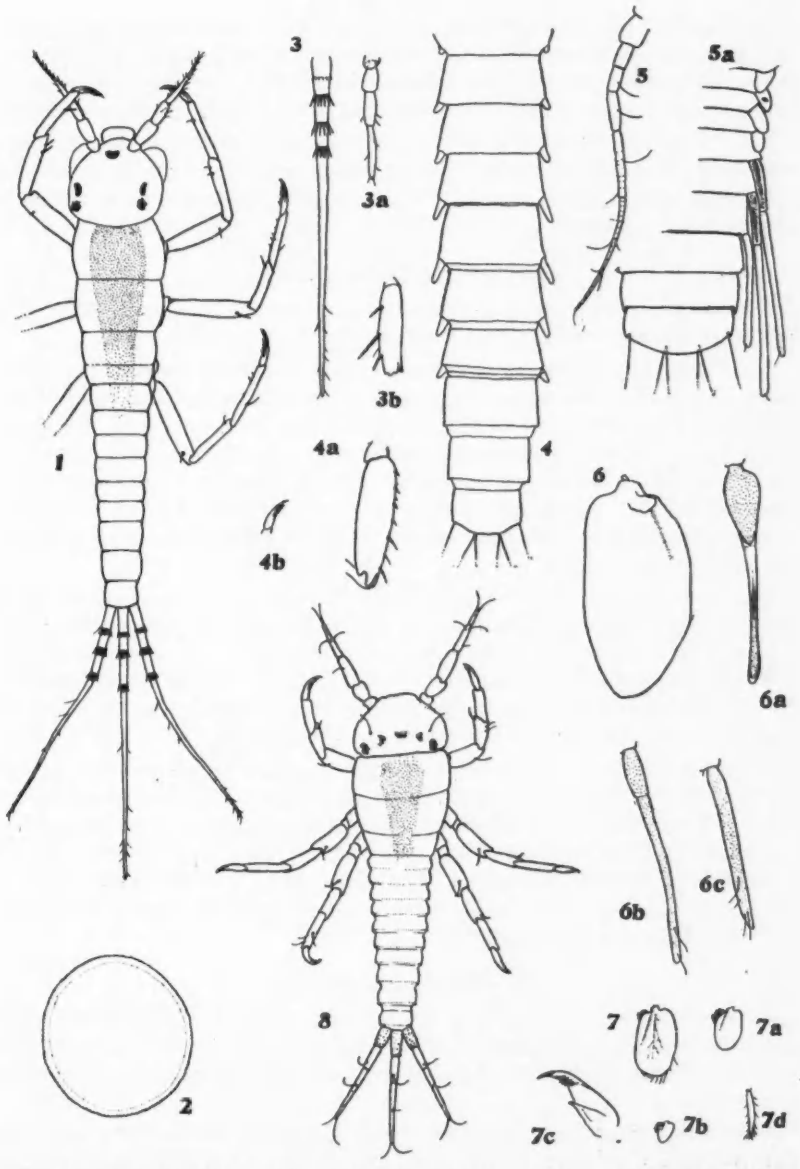

FIG. 12. *Isonychia bicolor. All mag. Y. 1, First instar. 2, Egg. 3, Second instar, lateral caudal filament; 3a, antenna; 3b, hind femur. 4, About fourth instar, showing gills; 4a, hind femur; 4b, tarsal claw.*

Stenonema fuscum. 5–6c, 7c, mag. Y; 7, 7a, b, 7d, mag. X. 5, Antenna; 5a, gills; 6, Third gill, ventral aspect; 6a, fifth gill; 6b, sixth gill; 6c, seventh gill. 7, Third gill; 7a, fifth gill; 7b, sixth gill; 7d, seventh gill; 7c, tarsal claw of foreleg.

Ephemerella subvaria. Mag. Y. 8, First instar.

of an organism radically changing its relation to its environment by growth. As it grows larger it has to adjust itself differently to the same flow of water, so that it is rather the relation of the environment to the organism which is important as a cause in metamorphosis than change in the environment itself.

This change in the tarsal claws is probably quite abrupt as was the case with *Epeorus* described above. An individual was not found which showed the change, but intermediate steps in the process were not seen in considerable material examined.

Heptagenia pulla Clemens

The last nymphal stage is described and figured by Clemens (1915). The first stage was not found in the present study.

A nymph (Fig. 13,–1, 1a, 1b) measuring 1.6 mm. in length showed the following points. The antennae have four well developed segments in the flagellum. The three caudal filaments, of which the central one is slightly longer than the others, are composed of 23 segments, the basal three of which are fused so as to be indistinguishable. Strong setae are present apically in Segments 6, 8, 10, 12 and 16. On Segment 14 there is a stiff hair only, so that probably Segments 15, 16, 17 and 18 were added at one ecdysis; this is probably the ninth or tenth instar.

Gills are present on Segments 2, 3, 4, 5, 6 and 7. On Segment 2 the gill is a mere bud; on Segments 3, 4 and 7 the gills are unsegmented filaments, a little longer than their respective segments. On Segments 5 and 6 the gills are about twice as long as on 4 and 7, and composed of two segments. By analogy with *Epeorus* and *Iron* the gills presumably appear on Segments 5 and 6 at an earlier ecdysis than on the other segments.

During subsequent development the gills assume a lamellate form and the tuft appears at the base of the gill, as shown for an intermediate stage in Fig. 13,–4, of Gill 5. All gills from 1 to 7 are now very similar except that 5 and 6 retain the terminal segment. This terminal segment is present on Gills 5 and 6 in the penultimate instar (Fig. 13,–3, 3a–c) and sometimes even in the last instar, as a mere vestige which has been generally overlooked in the published figures of this species.

Heptagenia hebe

A full-grown nymph was figured and described by Clemens (1915) under name *Ecdyonurus maculipennis*. This species was not reared in connection with the present study, but nymphal material was collected from the stones in the streams.

The earliest stage found (Fig. 13,–5 and 5a) shows the antennae with four well developed segments in the flagellum. The three caudal filaments are similar except for the slightly greater length of the median one. They consist of 16 well marked segments with strong setae distally on Segments 4, 6, 8, 10 and 12, and a terminal flagellum. It is probably about the seventh instar. Gills are present as long filaments on Segments 4, 5, 6 and 7, those on 5 and 6

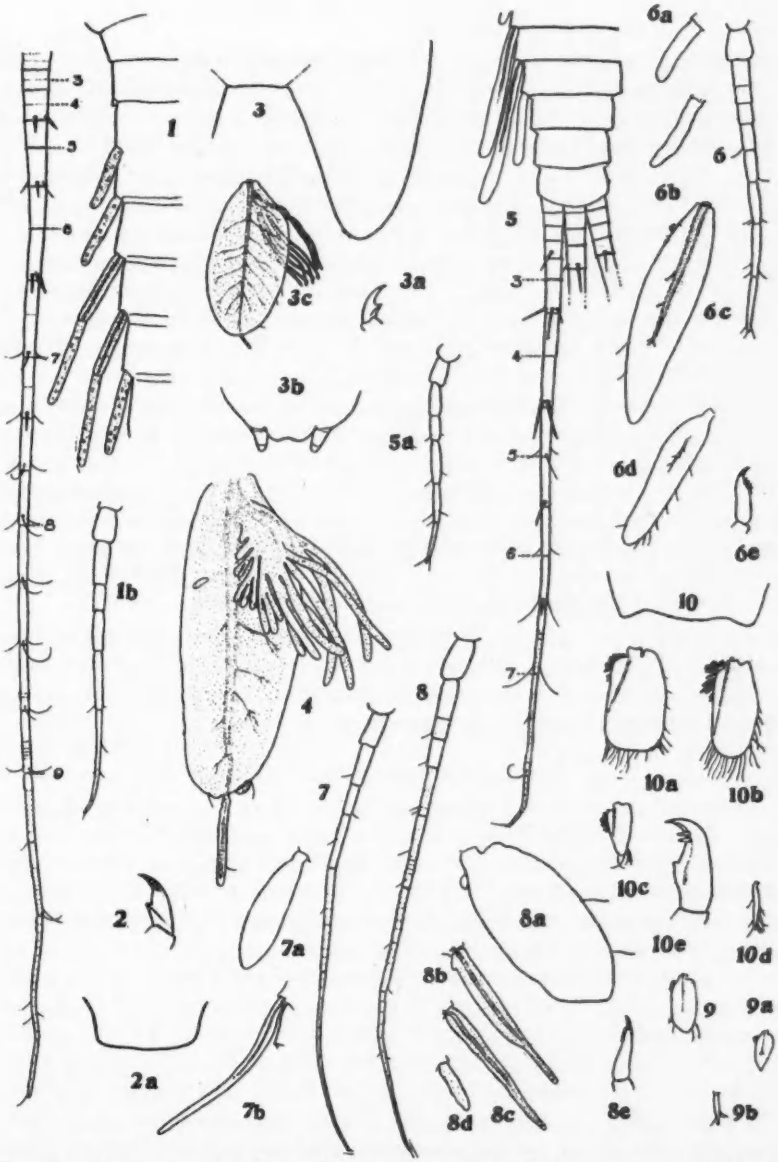

FIG. 13. *Heptagenia pulla. 1, 1a, 1b, 2 and 4, mag. Y; 2a, 3–3c, mag. X. 1, Ninth instar showing gills on Segments 2–7; 1a, lateral caudal filament; 1b, antenna; 2a, Mesothorax; 2, tarsal claw; 3, Penultimate instar, mesothoracic wing pads; 3a, tarsal claw; 3b, male genitalia; 3c, Gill 5. 4, Fifth gill.*

Heptagenia hebe. 5–6e mag. Y. 5, Seventh instar; 5a, antenna. 6, Antenna; 6a, 6b, 6c, 6d, Gills 1, 2, 5, 7 respectively; 6e, tarsal claw.

Stenonema tripunctatum. 7–8e and 10e mag. Y; 9–10d, mag. X. 7, Antenna; 7a, third gill; 7b, fifth and sixth gills. 8, Antenna; 8a, second gill; 8b, c, d, fifth, sixth, seventh gills; 8e, tarsal claw. 9, Gill 5; 9a, Gill 6; 9b, Gill 7; 10, Mesothorax; 10a, Gill 3; 10b, Gill 5; 10c, Gill 6; 10d, Gill 7; 10e, tarsal claw.

being about twice the length and of greater thickness than those on 4 and 7, but not segmented as in *Heptagenia pulla*. Here again, as in other cases referred to earlier, it seems extremely probable that there were gills present on Segments 5 and 6 before there were any on the other segments.

In a later stage (Fig. 13,–6, 6a–e) in which the antennae are composed of seven well differentiated segments, the gills are further advanced in development, and present on Segments 1 to 7. On Segments 1 and 2 they are short, on Segments 3 and 7 about twice as long as on 1 and 2, and on 4 they are slightly longer than on 3 and 7. On 5 and 6 they are longer than on any of the other segments but lack the terminal segment found in *Heptagenia pulla*. This seems to be a significant difference between the two groups in the genus *Heptagenia*, but of course may not prove to be a group character.

As development proceeds the gills grow broader, develop the tuft of filaments at the base, but otherwise do not change much and are very similar in form to one another. The gills on 5 and 6 keep ahead of the others in size, however, and the others retain their differences in relative length. It seems probable that in this form both segments of Gills 5 and 6 of *Heptagenia pulla* are represented in the corresponding unsegmented gills, and that the whole length of the gill has entered into the formation of the lamella. This would account for their relatively greater length in the ultimate nymphal instar.

In development of gills this species probably follows closely *Ecdyonurus forcipulus* Koll (see Gros, 1923).

The tarsal claws are pectinate as shown for the stage in which the antenna is of seven segments as shown in Fig. 13,–6e.

Stenonema tripunctatum Banks

The full-grown nymph is well described by Clemens (1915). In the earliest instar found the antennal flagellum contains four well differentiated segments (Fig. 13,–7). The anterior gills were lamellate and similar to the third of the left side which is figured in Fig. 13,–7a. Gills 5 and 6 (Fig. 13,–7b) are long and filamentous and similar to one another, and the gills of 7 are lacking entirely. It seems likely that in *Stenonema tripunctatum*, as in *S. canadense*, the gills of Segments 5 and 6 appear before those of any of the other segments.

The next stage found was the instar in which the antennal flagellum is composed of six well differentiated segments Fig. 13,–8. At this stage the gills on 1 to 4 are lamellate with the tuft of filaments beginning to form at the base. Gill 2 is shown in Fig. 13,–8a. The gills on 5 and 6 (Fig. 13,–8b and 8c) are long and filamentous, that of 5 being somewhat broader at the base and constricted toward the distal extremity. Gill 7 (Fig. 13,–8d) is not linear, but rather short, differing in this respect from *S. canadense*. The tarsal claw (Fig. 13,–8e) has the middle spine very much developed and the others arranged on each side of it as two rows of pectinations.

In a later stage the antennae have about 13 segments differentiated in the flagellum. The gills of this instar on Segments 5, 6 and 7 are illustrated in Fig. 13,–9, 9a and 9b. Gill 7 is lanceolate and lacks the tuft of filaments

at the base. Gill 6 is lamellate and has dropped the terminal portion whose former position is marked by a small tubercle at the apex of the gill. Gill 5 is lamellate and shows no sign of the linear tip; it has metamorphosed so that it is similar to the anterior gills. Gills 5 and 6 both have the tuft of filaments at the base developed at this stage.

In a still later stage (Fig. 13,–10, 10a–e), in which the antennae have about 19 well developed segments in the flagellum the gills are very similar to those of the full-grown nymphs. The main change now takes place in the greater size of the gills and the greater complexity of the tuft of filaments at the base. In this stage the wing pads (Fig. 13,–10) have been developing through three or four instars and the claws (Fig. 13,–10e) have changed somewhat, the change being mainly in the reduction in the number of pectinations. In one series there are two, and in the series of the opposite side, but one angular projection which may be a different structure entirely from the claws which form the pectination. Gill 7 has not changed its form. Gill 6 has lost the vestige of the terminal part, is triangular in shape and shows a greater development of the tuft of filaments at the base. Gills 3 and 5 are similar to one another and have a truncate posterior border.

Stenonema fuscum Clemens

The full-grown nymph is described by Clemens (1915) and also by McDunnough (1933). This form was not studied in great detail, but three stages are figured, which throw some light on the development of gills in this group. At the stage (Fig. 12,–5, 5a) in which the antennal flagellum contains four segments proximally, and the nymph is about 1 mm. in length, gills are present on Segments 1 to 4 as short rounded projections varying slightly in length, but in general being about equal to the length of the corresponding abdominal segment. Gill 3 is slightly longer than the other gills of the anterior series; Gill 7 is long and unsegmented, similar to the gills of the same segment in *S. canadense*. The gills on 5 and 6 are similar to each other and are divided into a short basal segment and a terminal segment which is nearly three times as long as the basal one. These gills are more like those of *Iron*, *Epeorus* and *Heptagenia pulla* than those of *Stenonema canadense* in this respect. Their greater length and the presence of the trachea in the basal segment suggest that these gills appeared earlier than the other gills, probably at the second instar. The tarsal claws are similar to those of *S. tripunctatum* in possessing two rows of pectinations, one on either side of the central claw.

Fig. 12,–6, 6a–c, taken from an individual 1.7 mm. long in which nine segments were differentiated in the antennal flagellum, forecast the probable fate of each segment of the fifth and sixth gills. The basal portion has become expanded (6a), the content has been constricted off at the intersegmental region, so that at the next instar the distal segment will be dropped altogether and the basal segment will form the ultimate gill lamella as in *Epeorus*. Gill 7 is unsegmented and remains practically the same throughout development, and in the full-grown nymph is referred to as a vestigial gill.

Fig. 12,–6 shows the tuft of gill filaments forming at the base of the lamella of Gill 3. Gills 1–4 are all lamellate and similar to Gill 3, although varying somewhat in size. In Fig. 12,–7, 7a–d are shown Gills 3, 5, 6 and 7 at a later stage (antennae 16 segments) when Gills 5 and 6 have lost their distal segments and are similar to Gills 1 to 4. Gill 7 retains its simple and lanceolate character.

Leptophlebia debilis Walker

The full-grown nymph of this species is described by the author (Ide, 1930).

Some of the younger stages of this species were found. In the stage in which there are eleven segments in the antennal flagellum, the gills (Fig. 4,–27 to 29) are very long, slender filaments, showing the beginning of the inner or upper ramus of the gill. This ramus, which later becomes nearly as long as the primary ramus, is at this stage a mere bud at the base of the gill. A branch of the trachea runs into it. Undoubtedly in an earlier stage the gills would be simple lanceolate filaments without the side branch. A European genus, *Paraleptophlebia*, has been figured in this stage (Lestage, 1916).

Discussion and Conclusions

A study of the life histories of these nymphs has brought out some points which will be discussed under five headings:

(a) Origin of segments in the antennae and caudal filaments.

(b) Variation in the number of caudal filaments present in mayflies.

(c) Metamorphosis of claws and mouth parts.

(d) Appearance and metamorphosis of gills.

(e) Metamorphosis in general.

Origin of Segments in the Antennae and Caudal Filaments

Both antennae and caudal filaments show some points regarding the formation and addition of segments during growth, which should be noted. The antennae and filaments roughly maintain their length relative to the size of the instar, with a few exceptions (see *Baetis*).

In the very small nymphs a very flexible flagellum is satisfactory, but as the nymph grows in size the basal part of the flagellum must become more solid and rigid for some distance, the intermediate part must be flexible, accomplished by the production of segments or joints which allow of movement and yet are rigid enough to give support. Rigidity and increase in size at the base of the flagellum is accomplished by the compression of the segments until they are much thicker than long, and then by fusion to form a rigid base. This gives the appearance of a growing point at the base of the flagellum, the segments originating by a splitting of the original segments into two or four parts, as suggested by Lubbock, (1864 and 1867). The development of the flagellum of the antennae and caudal filaments in *S. canadense* and others demonstrates that the segments do not arise in this way, but rather by a differentiation of segments from the proximal end of the

unsegmented flagellum, the new segments being added distal to those formed in the previous instar. The clearest evidence for this is found in an examination and comparison of the terminal unsegmented part and the adjacent segments of the antennae in early instars of *S. canadense*. The terminal part will be seen to be divided into minute subsegments of about equal volume (Fig. 6,–2a). As we pass proximad a place is found where these subsegments quite suddenly become noticeably shorter and appear to be more immovably united to one another. This point is usually roughly shown by the appearance of one or several hairs a little proximad of it. About ten of these subsegments make up one segment and what is being examined then is a segment in the process of formation. Next to this region proximally is a well formed segment in which, however, the original subsegments of which it is composed are clearly distinguishable. Examination of more proximal segments in later instars shows that the vestiges of these subsegments are lost entirely nearer the base.

The origin of segments in the caudal filaments takes place in the same way except that here the segmentation is still more complex. The small subsegments of the terminal part fuse in numbers to form a segment. Then at the base of the filaments in later stages the segments themselves become indistinguishably fused with one another to form a rigid base. Further, the segments formed are not uniformly definite, even at the time of their appearance. In *S. canadense* one segment makes its appearance at the first ecdysis. At the next ecdysis one segment is added and a stout seta appears distally in Segment 4. At the next ecdysis two segments are added, the proximal one (Segment 6) bearing apically a stout seta similar to that on Segment 4. The separation between the fifth and sixth segments is not nearly as well defined as that between 6 and 7, and thus, as development proceeds, Segments 5 and 6 will soon become fused so as to be indistinguishable and this fusion will not take place between 6 and 7 till a later period in development. Segments 13 to 16 are produced also at one shedding; now the joint between 12 and 13 and that between 16 and 17 are each much more prominent than the joint between 14 and 15, and the joint between 14 and 15 is in turn more definite than the joint between 13 and 14 and that between 15 and 16. Thus, as development goes on and these segments begin to fuse and enter into the basal part of the filament, the junctions between 13 and 14, and between 15 and 16 will disappear first; then the joint between 14 and 15, and finally the joint between 12 and 13 and that between 16 and 17.

The limit of the segment is determined in the undifferentiated terminal part of the flagellum. Usually these joints form in a definite way in regard to the position of the hairs and setae, so that these structures are in the distal part of the segment. Sometimes, however, something goes wrong and the segment limit or joint does not fall at the right place, and the hairs are not present distally in the proper segment. For example in the third instar of *S. canadense* there is, with very few exceptions, a strong seta distally in Segment 4 of the caudal filaments. In an occasional specimen, however, (and

in these it is usually in but one of the filaments) the seta is absent from Segment 4 but is present distally in 5. In this case, however, Segment 4 is much shorter than the normal, and Segment 5 is also rather short, showing that the joint had occurred at the wrong place. Even so, the circlet of spines forms distally in both 4 and 5. From this it would appear that the position of the setae in the caudal filaments is more fundamental than the position of the segments. In this connection Lubbock noted that the dark bar on the caudal filaments of *Cloeon dimidiatum* retained its position in the distal third throughout the nymphal period, in spite of great changes in the position of the segments.

In the caudal filament (Fig. 5,–30, 30a) of a full-grown nymph of *Ephemera*, there are a number of intimately fused segments at the base, but many of them are still distinguishable. As the segments are examined distally they become more distinct and then show a division into two, which is at first very indistinct. The suture dividing these secondary segments forms a sinuate line rather than the straight line between the original or primary segments. Still further out on the tail the primary segments show division into four, or a subdivision of the two halves, also by sinuate lines. At first these sutures, which might be termed tertiary sutures, are indistinct, but further out they become very distinct so that each primary segment is delimited by a straight suture from adjacent primary segments and divided into two secondary segments by a sinuate line, and these secondary segments are each divided into two tertiary segments by a sinuate line or suture. In development, each primary segment represents the addition to the caudal filament at one moult. The basal primary segments are undivided; a few adjacent segments are divided into two secondary segments; those formed subsequent to the eighth instar are composed of four tertiary segments. The caudal filament (Fig. 5,–30, 30a) does not show all the segments formed owing to loss through fusion at the base. And furthermore, as pointed out above, in the process of fusion and disappearance of the intersegmental sutures, the tertiary ones disappear first, then the secondary and finally the primary. If all the segments remained separate and distinguishable it would be possible to count the number of primary segments in the filament and thus have the number of instars through which the nymph has passed. This number, together with the well known subimago and adult instars, would be the full number passed through by the individual. In the subimago instar there is apparently a freeing again of at least some of the segments fused during nymphal life. In Fig. 5,–30 to 34c, the corresponding segments in full-grown nymph, subimago, and male and female imagos are similarly designated. The determination of these homologies was made possible by the finding of nymphs about to emerge, which showed the enclosed segments of the subimaginal caudal filaments, and also by the fact that the imaginal segments were apparent within the subimaginal cuticle. The segments, it will be seen, correspond very well with those of the previous stage, the main difference being an increase in length of the segments. In the case of *Ephemera*, the pigmentation at the joints helped greatly in working

out the homologies in the last three instars. Fairly well out on the tail, as mentioned above, each primary segment is composed of four tertiary segments, these being separated not by straight lines as are the joints between the primary segments, but by sinuate lines. The subimaginal tail developing within the nymphal tail near the end of the last nymphal instar fortunately shows a difference in pigmentation at the junction of the segments. This indicates that the segments correspond. Between the primary segments there is very little pigmentation, while between the secondary and tertiary segments there is very dark pigmentation, resulting in the middle third of the caudal filament in the formation of a light band followed by three dark bands, then another light and three dark bands, and so on. This plan can be followed well out on the tail, a few segments only, near the tip, not showing distinctly the grouping into four by the pigmentation. Towards the base the dark rings between the tertiary segments disappear first and then finally those between the secondary segments. There is a narrow constriction at the base of the subimago caudal filament, designated X, which can be fairly accurately placed and so serve as a starting point in a count of the segments.

In *Stenonema* also the segments are apparently added in fours at each ecdysis through most of the nymphal life. Here the segments are also nicely grouped in fours not by the difference in character of the joints themselves as was the case with *Ephemera*, but by the presence of setae at the apices of the segments and also somewhat by the pigmentation. In the basal part the primary segments from the fourth on are armed in the middle with very stout spines in a single row. Farther out the spines disappear, but they have been present far enough out on the tail to show that the method of addition of the segments is apparently the same as in *Ephemera simulans*. In the basal third of the filament the apices of the primary segments are not armed with thick, stout setae, but with longer, thinner ones and possess a band of pigment. Following out the tail to the region of the tertiary segments it is seen that the pigmented band disappears from the region between the primary segments and is now found on the line dividing the tertiary segments. After the disappearance of the stout setae in the middle of the primary segments distally in the caudal filament there is nothing to distinguish them, so that a primary segment is represented by two secondary segments which were added at one ecdysis and are similar to one another as far as could be ascertained.

Each of these secondary segments is divided by a suture into two tertiary segments with darkened bands between. Basally the segments are fused together so that first the tertiary joints disappear, leaving two secondary segments only to each primary, and then the secondary joints disappear, leaving only the primary joints, which eventually disappear also.

In *Ephemera* the change in the caudal filaments from the subimago to the imago stage was the most difficult to understand. The imaginal filament was much longer than the corresponding subimaginal filament, and it was surprising to find that in spite of this there were fewer segments present and

that the lengthening had been caused by the elongation of segments. At first it was concluded that the decrease in the number of segments had been caused by fusion of segments at the base, but an individual which was about to change into the subimago showed that this was not the case. In this individual (Fig. 5,–31a) the reduction was caused by the dropping of the twelve distal segments of the nymphal filament. Other specimens of this species examined later showed the same phenomenon. The process is the same as that taking place in the antennae between the last nymphal instar and the subimago as described by Lubbock (1867) for *Cloeon dimidiatum*.

Variation in the Number of Caudal Filaments Present in Mayflies

The number of tails present in mayflies has always been an interesting question. Several different conditions seem to exist.

1. Nymphs hatch with three caudal filaments of about equal length, and retain these throughout nymphal and adult life, *e.g.*, *Ephemera*, *Ephemerella*.

2. Nymphs hatch with three caudal filaments of about equal length, retain these throughout nymphal life, and lose the middle tail in the subimaginal and adult stages, *e.g.*, *Stenonema*, *Heptagenia*.

3. At hatching, there are two well developed lateral filaments and a reduced median, one which is further reduced in early nymphal life, and remains so throughout the later stages, including the subimago and adult, *e.g.*, *Iron* and *Epeorus*.

4. Nymphs hatch with two well developed lateral filaments, but no median one. The median one grows out during nymphal life though not attaining the length of the lateral ones. In the subimago and imago the median filament is dropped and the lateral ones retained, *e.g.*, *Baetis posticatus* (Murphy 1922), *Baetis vagans* and *Cloeon dimidiatum* (Lubbock).

5. There is perhaps another condition too in which there are only two caudal filaments at hatching (the two lateral ones or cerci), the third or median filament not being present or making its appearance during subsequent stages including the subimago and adult. *Pseudocloeon* seems to illustrate this type but since first stage nymphs have not been examined it may turn out that *Pseudocloeon carolina* has a reduced median filament which is dropped early in development as was the case with *Iron* and *Epeorus*.

The two-tailed condition in nymphs seems to be associated with life in very rapid water (*Iron*, *Epeorus* and *Pseudocloeon*) where obviously the tails are not used for swimming and therefore the addition of a middle tail could be of little advantage.

The lateral caudal filaments are generally conceded to be the cerci, appendages of the eleventh segment, and the median caudal filament the greatly elongated telson.

The evidence available seems to indicate that the three-tailed condition is primitive within the order Ephemeroptera, the two-tailed condition having arisen secondarily by the dropping of the median filament. The great

majority of species have three caudal filaments in the nymphal instars, some of which drop the median caudal filament in the subimago and adult, and others retain it. Some of the nymphs which have but two tails present have a vestigial median filament in the early instars and many adults which apparently have but two filaments have a rudiment of the median present. Apparently the median filament is a very variable structure, being present in the adults of some species and absent in the adults of other species, even in the same family. In order to consider the two-tailed condition primitive, the assumption would have to be made that the median tail had been independently developed in several families, which is extremely improbable. The logical conclusion is that the three-tailed condition is primitive and that mayflies had three-tailed insects as ancestors.

Metamorphosis of Claws and Mouth Parts

In *Stenonema* the molar surface of the mandible is relatively undeveloped at the time of hatching, and the other mouth parts (Fig. 6,–1a–c) also differ greatly from their final condition in the half-grown or full-grown nymphs. The canine teeth on the mandibles of each side are directed medially so that they probably function in ingestion. Later these canine teeth are directed anteriorly and probably do not take part in ingestion in the same way as formerly, that function being taken on by the molar surface. This suggests that the canine teeth function as early larval organs.

An examination of stomach content was not made, but, assuming that the young nymph eats the same organisms and materials as the older nymph, we would expect that a different mechanism would be required in a small and large nymph in order to capture effectively and take in the same organisms. The relation of food organism and nymph has altered not by any change in the former, although this may occur also, but merely by the increase in size of the nymph which necessitates morphological changes to meet the altered environmental relation thus created.

A series of tarsal claws of *Epeorus humeralis* is shown (Fig. 10,–2c, 9, 10, 11, Fig. 11,–13b and 14c), illustrating the metamorphosis which takes place in these parts. There is a gradual increase in the number of claws up to the stage illustrated by Fig. 10,–11, at which time there is a reduction in the number of claws as indicated by the new claw developing within. The new claw has changed its form very abruptly. There is from now on one great curved claw with some of the other claws present as a row of pectinations on the great claw. This abrupt change in the type of tarsal claw coincides with a probable change in function of the claw as described under *Epeorus humeralis*.

The nymph throughout its life cycle must be adapted to hold itself in position in a strong current of water. Obviously the very small nymph will hold to very minute irregularities on the surface of the stones. The many-pectinated or many-clawed last tarsal segments of the early stage nymphs are adapted to clinging to such a surface. With growth these claws increase in number up to the stage shown in Fig. 10,–11, when there is a sudden meta-

morphosis of the claws, to form the type shown in Fig. 11,–13b, 14c, with one claw greatly elongated and hooked and the four others present as pectinations along its lower surface. The nymph by this stage is much larger than at hatching (about 6 mm. long), so that the minute irregularities on the surface of the rock will no longer provide a suitable hold, but irregularities of a larger order, and therefore less in number relative to the nymph's immediate environment, will be used. For this purpose the new type of claw will be much more satisfactory since it will hook around these larger irregularities and projections. This sudden metamorphosis of the claws also coincides with the time at which the gills reach the substratum and develop along their lower edge combs of posteriorly directed setae, which function in holding the nymph in place in the current of water. It is just at this time in their life that the nymphs of *Epeorus humeralis* drop down-stream out of the very rapidly flowing water into the more slowly flowing water below the rapids (Ide 1935).

Appearance and Metamorphosis of the Gills

The gills appear as prolongations from the postero-lateral angles of the segments. In *Ephemera* and *Isonychia* they appear simultaneously on Segments 1 to 7 inclusive. In *Stenonema canadense*, *Epeorus humeralis* and *Iron pleuralis* the gills of Segments 5 and 6 appear several instars earlier than those on 1, 2, 3, 4 and 7 and this is probably true for *Heptagenia pulla*, *H. hebe*, *Stenonema tripunctatum* and *Stenonema fuscum*. The gills of 5 and 6 in these forms grow out as long filaments which later metamorphose into lamellae similar to the anterior gills which have developed into lamellae directly, never having the filamentous form. Gill 7 in some forms becomes filamentous, *e.g.*, *Stenonema canadense*, or may develop as a lamellate plate as in *Heptagenia*. The metamorphosis of Gills 5 and 6 from filaments to lamellae may take place in two ways. If these gills are composed of two segments as in *Epeorus humeralis*, *Iron pleuralis*, *Heptagenia pulla* and *Stenonema fuscum* the change involves only the basal segment which becomes expanded and lamellate and the distal segment remains unchanged and is eventually dropped. If the gills are unsegmented, as in the case of *Heptagenia hebe*, *Stenonema canadense* and *Stenonema tripunctatum*, the whole gill enters into the formation of the lamella. In the former process the result is a gill with a rounded or truncate posterior border while in the latter the result may be a pointed gill as in *S. canadense*. All gills appear first as uniramous structures, but early in their growth most of them develop a secondary medial ramus which may take several forms. In *Ephemera* and *Leptophlebia* it grows out and becomes similar to the primary ramus though not attaining its length or complexity. In *Epeorus*, *Iron*, *Stenonema* and *Heptagenia* the secondary ramus takes the form of a plumose tuft of filaments. The secondary ramus may be absent from all the gills as in *Baetis*, or may be absent from one or more gills, *e.g.*, Gill 7 in *Stenonema* which remains uniramous throughout development.

The gills contain tracheae and some of them at least are provided with muscles which move them to and fro in the water. Fig. 11,–15 represents

a young nymph of *Iron pleuralis* in which muscles pass from a suture on the venter of the segment (line separating the zygosternum into two parts, the sternum and the limb bases) to the proximal part of the gill. This muscle does not continue into the gill even when the latter is a two-segmented structure. Durken (1907) has discussed the musculature of the tracheal gills of ephemerids and has come to the conclusion that the gills are dorsal or notal structures, although he does not homologize them with wings as some others have done. The more generally accepted view is that they represent abdominal legs (Snodgrass, 1931, and Spieth, 1933).

Metamorphosis in General

The number of instars in mayflies is very great compared with that in other insects, a phenomenon probably inherited from ancestors and retained because of the special requirements of this group. At each of the moults there is some increase in size of the nymph but the moult seems primarily for the purpose of changing the morphological structure. Although the nymph is increasing slowly in size, some structures such as mouth parts, wing pads, gills and genitalia are changing radically in size and complexity by differential growth rate of the parts. Some of this metamorphosis is merely concerned in perfecting the adjustment of nymphal structure to the changing conditions. Examples are the changes in mouth parts, eyes, gills and tarsal claws. Other metamorphosis is concerned with the development of such adult structures as the wings and external genitalia.

The cuticle of insects is very elastic so that it seems unlikely that so many moults could be concerned primarily with increase in size. Many other insects grow to much larger size with but four or five moults. The reason for the great number of moults seems to be in the constant adjustment which the nymph must make to its physical environment (*e.g.*, food, rapid flow of water). Every change in external structure, even to the addition of a seta or hair, can be accomplished only by a moulting of the old skin and the emerging of the new stage adapted to its brief duration as was each stage prior to it. A long resting period is precluded in these forms because of the necessity of their being maintained in a rapid current and actively feeding all the time. Consequently any large structure does not form completely at one moult, but requires several, as for example, in the formation of the mandibular tusks in *Ephemera*. Very abrupt changes are rather the exception than the rule in this group and most of these occur when the insect leaves the water, at which time the environment is radically changed. Examples of such changes are found in the development of turbinate eyes in some forms, in the great reduction in size of the antennae, often in the dropping or aborting of the median caudal filament or in the shortening of the filaments as was demonstrated for *Ephemera simulans*. There is a striking difference in the time required to develop a structure such as a caudal filament or an antenna and the time required to get rid of the same structure. By a process of differential growth, which probably follows closely the exponential curve, the structure is gradually developed through several instars but it may be eliminated in much less time, at one ecdysis.

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CYTOLOGY OF THE BLOOD OF NORMAL MINK AND RACCOON

I. MORPHOLOGY OF MINK'S BLOOD[1]

By Arnold H. Kennedy[2]

Abstract

The morphology and number of blood cells occurring in normal mink and raccoon have been determined in order to establish normal standards for various periods during the life of these animals. These standards are desirable for comparative purposes when determining the extent of pathological blood changes which may take place during the course of nutritional or parasitic diseases.

Normoblasts and poikilocytes were observed in mink's blood. Reticulocytes were also common. Forms of lymphocytes ranging from 6.5 by 6.0 μ to 11.3 by 9.5 μ are found. The monocytes range in size from 11.0 by 9.5 μ to 15.5 by 13.0 μ. In the neutrophile, band-formed nuclei are common, comprising from two to eight per cent of the total number. Their sizes range from 11.5 by 11.0 μ to 16.0 by 12.0 μ. Numerous, well defined, reddish-brown granules are found in the cytoplasm of the eosinophile. In the eosinophile also, the lobes of the nuclei are less lobulated and closer together than in the neutrophile. The eosinophile may range in size from 11.5 by 10.0 μ to 16.0 by 15.0 μ. The cytoplasm of the basophile contains from 22 to 65 large prominent granules, which stain dark blue to purple. Granules which are almost indiscernible are also present in the cytoplasm. The basophile ranges in size from 11.5 by 11.0 μ to 14.0 by 12.0 μ. Large masses of platelets ranging in size from 1.5 by 1.0 μ to 2.5 by 2.0 μ are often observed in smears from mink's blood.

Introduction

It was decided that the morphology and number of blood cells occurring in the normal mink and raccoon should be determined before attempting to record data obtained from blood examinations during the course of investigations of the nutrition and the parasites of these animals. This series of papers is based on data obtained from examinations of the blood of normal mink and raccoon. Each paper covers a portion of the work and the series contains descriptions of the cellular morphology and photomicrographs of each cellular element for comparative purposes. Statistical tables have been prepared showing the blood counts for the various age groups of each species.

No references relating to the blood of these animals could be found in the literature. In determining the extent of pathological blood changes, normal standards for various periods during the animal's life are desirable for comparative purposes. It is hoped, therefore, that the material contained in these papers may save some other worker a considerable amount of tedious work and serve as standards for the blood of normal mink and raccoon.

Materials and Methods

The animals used for these studies were selected from breeding stock and their progeny kept at the Ontario Government Experimental Fur Farm. Animals under a year old were bled at various times throughout the year. The data obtained were classified according to the sex and age of the animals.

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Contribution from the Ontario Government Experimental Fur Farm, Kirkfield, Ontario.
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Animals over a year old were considered as adults. The ages of adult mink varied from over one, to four years; the ages of adult raccoon from one to eight years. Blood counts taken from pregnant females or females suckling young were not included.

Blood examinations were not made on animals less than three weeks old because female mink and raccoon resent interference with their young and consequently may destroy them if they are handled at birth or soon after.

All animals were in good health and nutrition. No disease or parasites appeared among them during the course of the work. The housing arrangements and feeding and environmental conditions generally were uniform for each species, and were those accepted as being adequate for ranch-raised mink and raccoon. Standardized equipment was used throughout the investigation.

Extraction of Blood

The mink were held in restraint by having an assistant grip them firmly around the neck with one hand and hold the foot from which the blood was to be extracted with the other. A small net and thick leather gloves were used in catching and handling the mink.

The raccoon, excepting those under two months old, were caught in a net, and a large funnel about two and a half feet long, twelve inches across at one end and tapering to about three inches at the other, was placed over the head. The raccoon was forced into the funnel, from which a hind foot could readily be drawn out and held in position for bleeding without fear of the animal biting.

The most suitable place for extracting blood from the mink is similar to that described for the fox (1), *i.e.*, the blood vessel found in the integument between the toes.

The site found most suitable for obtaining blood from the raccoon is further up on the foot, between the metacarpal bones and close to one of them The metacarpal blood vessel is not visible but a plentiful supply of blood is readily obtained by puncturing the skin with a small sharp pointed scalpel (No. 11. Bard Parker Blade).

Before obtaining samples from these locations the fur was clipped with a pair of scissors and the area cleaned with alcohol. After the puncture was made the first few drops were rejected in order to avoid the local effects of manipulation and any possible contamination with loose tissue.

Enumeration of the Blood Cells

For the enumeration of the red blood cells, the blood was diluted 200 times in a standardized Trenner diluting pipette (1–101). Toisson's fluid was used throughout the investigation for diluting purposes. A haemocytometer certified by the United States Bureau of Standards was used for counting the red blood cells. This has Levy-Hausser counting chamber and improved Neubauer double ruling. The enumerations were based on the examination

of 100 squares, each 1/400 mm. long. These were composed of six groups of 16 and four additional squares. The groups were selected from the milled area of the counting chamber. No attempt was made to select any given group.

The average size of the red blood cells was determined by the use of the so-called halometer or eriometer, which gives a direct measurement of the red blood cell by the diffusion of light. The instrument was first described by Pryce (2) (second method).

The white blood corpuscles were counted in the standardized haemocytometer and sampled with a Trenner diluting pipette (1–20). The diluting fluid was 0.3% glacial acetic acid in aqueous solution. The white corpuscles covering one sq. mm. in each quarter of the milled area were counted. The proportions of the different types of white blood cells were determined by classification of 200 consecutive white cells from a blood film. Their respective numbers were then estimated from the total number found in a cubic millimetre of blood.

The Morphology of the Blood Elements

The technique used in making and staining the films for studying the size, shape and appearance of the various corpuscles in detail and for making differential counts of the white blood cells was that described by Kennedy (1).

Morphology of Mink Blood

Red blood cells. The red blood cells when stained with Hasting's stain give a pink to rose-red color. The centre stains paler than the periphery owing to the biconcave formation of the cell. In young mink, the cells appear to stain a lighter color.

The average diameter of the red blood cells is 7.8 μ; the maximum size is 9.5 μ and the minimum 6.0 μ. Normoblasts were found in mink of all ages but were most frequently seen in mink three and four months old and were also quite common in the adult mink. One to six normoblasts have been recorded in an examination of 200 leucocytes.

Reticulocytes are common in mink blood; anisocytosis was observed in young suckling mink; poikilocytosis was observed in mink of all ages.

Lymphocytes (Plate I, Figs. 1 and 2). Large and small forms of lymphocytes are found in mink blood, but intermediate forms ranging in size from the small to the large make it impossible to separate them into two groups. The smaller forms are more numerous than the larger. Their nuclei, which are usually round, oval or slightly indented in shape, comprise the larger portion of the cell. A narrow band of cytoplasm may surround the nuclei or a small crescent-shaped portion may be visible. In the larger forms, the nuclei are round, oval, bean-shaped, or slightly lobed, situated in the centre or to one side of the cell, and are usually surrounded by a comparatively wide band of cytoplasm.

The nucleus of the smaller forms appears more compact and stains a dark blue to purple color. In the larger forms heavily staining nuclear masses are separated by pale narrow bands. The cytoplasm of the smaller lymphocytes stains a pale blue color while the cytoplasm of the larger forms stains more deeply and shows a clear gray-blue color.

The average size of the lymphocyte is 11.3 μ by 9.5 μ. The maximum size is 15.0 by 12.0 μ, and the minimum size is 6.5 by 6.0 μ.

Monocytes. (Plate I, Fig. 3). The nucleus may be bean-shaped, U-shaped or kidney-shaped, with the convex side usually facing the margin of the cell. The nucleus stains a dark blue color, with portions of the dark blue stained chromatic material being divided by bands staining a light greenish to gray-blue color, similar to that of the cytoplasm. The cytoplasm has a slightly roughened appearance and no granules were observed in it.

The average size of the monocyte is 13.0 by 10.0 μ. The maximum size of the monocyte is 15.5 by 13.0 μ and the minimum size is 11.0 by 9.5 μ.

Neutrophiles. (Plate I, Figs. 4 and 5). In appearance, the neutrophile of the mink is not unlike the neutrophile of the fox (1). The nuclei stain a deep blue to a purple color and are segmented, having from two to seven lobes, five being the average. The lobes may be quite widely separated and connected by a narrow filament. Portions of the nuclear chromatin stain a dark blue color and are separated by bands staining the same color as the cytoplasm. The cytoplasm stains a very faint sky-blue color and contains a large number of very small reddish-brown granules which in many instances are difficult to observe, so that the cytoplasm has a more or less clear appearance.

Band-formed nuclei are quite common, comprising from two to eight per cent of the total number found. These forms are more common in the younger mink.

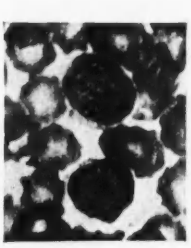
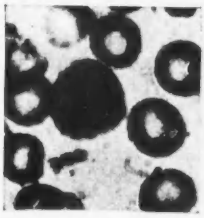
Metamyelocytes were observed and approximate from 0.5 to 2.0% of the total number of neutrophiles.

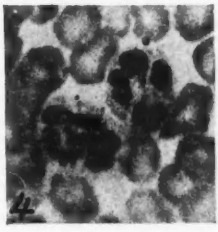
The average size of the neutrophiles is 14.5 by 12.5 μ. The maximum size is 16.0 by 12.0 μ and the minimum size is 11.5 by 11.0 μ.

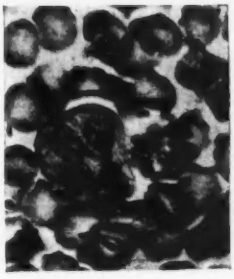
Eosinophiles. (Plate I, Figs. 6 and 7). The nuclei of the eosinophile stain lighter blue than those of the neutrophile of mink blood. The lobes are less lobulated and closer together, not being separated by visible chromatin filaments. Since the chromatin masses are not so pronounced, or so prominently divided by lighter staining bands, they give a finer, more granular appearance than the chromatin masses of the neutrophile and basophile.

The cytoplasm stains so lightly that it is almost indistinguishable. Numerous, well defined, dark red to reddish-brown granules, measuring approximately 0.5 μ in diameter, loosely fill the cytoplasm and may obscure portions of the nucleus. The margin of the cell is regular in outline.

The average size is 14.5 by 13.5 μ. The maximum size is 16.0 by 15.0 μ and the minimum 11.5 by 10.0 μ.





FIGS. 1 and 2. *Lymphocytes.* FIG. 3. *Monocyte.*
FIGS. 4 and 5. *Neutrophiles.* FIGS. 6 and 7. *Eosinophiles.*
FIGS. 8 and 9. *Basophiles.* *Mag. 14 X.*

Basophiles. (Plate I, Figs. 8 and 9). The nucleus may be U-shaped or highly lobulated and is then not unlike the neutrophile in form and structure although the lobes are more closely attached to one another. The nucleus stains blue to light purple and is but very little darker than the cytoplasm. The chromatin masses stain more deeply and are separated by narrow pale bands. The cytoplasm stains a pale blue color and has an almost glassy appearance. It may contain from 22 to 65 large prominent granules up to 1 μ in size. The granules appear to stand out from the surface of the cytoplasm and stain dark blue to purple. Granules which are almost indiscernible are also present in the cytoplasm.

The average size of the basophile is 14.0 by 12.0 μ. The maximum is 16.0 by 13.0 μ and the minimum is 11.5 by 11.0 μ.

Degenerated forms of cells found in mink blood. Cells which could not be given a definite classification are placed under this heading. These cells are probably old and degenerate forms which have lost their characteristic identity. It is thought that the majority of them belong to the monocytic and lymphocytic classification. These cells were found more frequently in older mink. The majority of them were irregular in outline, stained deep blue to greenish-blue, and presented a vacuolated and frayed appearance, with no distinguishing characteristics. The so-called basket cells were observed in all classes of mink but appeared more frequently in the adults.

Platelets. Large masses of platelets were often encountered in the blood smears. When massed together they lost their individual identity. When single platelets were observed they were round, oval or irregular in outline. The cytoplasm stains a very light blue and contains fine, deep-blue staining structures, irregular in outline and usually situated in the centre of the cytoplasm in the form of a crescent or circle. Again these structures may be broken up and appear as deposits of fine dust-like particles.

The average size of the platelet is 2.0 by 1.5 μ. The maximum size is 2.5 by 2.0 μ wide and the minimum 1.5 by 1.0 μ.

Acknowledgment

The writer wishes to express his appreciation to Dr. Ronald G. Law, Director of the Ontario Government Experimental Fur Farm, Kirkfield, Ontario, for his interest and assistance throughout the course of the work, and also to thank Mr. Allan Jewell of the same institution for his kind co-operation in the handling of the animals and in the preparation of the manuscript.

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CYTOLOGY OF THE BLOOD OF NORMAL MINK AND RACCOON

II. THE NUMBERS OF THE BLOOD ELEMENTS IN NORMAL MINK[1]

By Arnold H. Kennedy[2]

Abstract

The numbers of lymphocytes, monocytes, neutrophiles, eosinophiles and basophiles in the blood of normal mink of various age groups are listed. Data are given showing the maximum, minimum and average numbers of these types of cells. Similar data are supplied for white and red blood corpuscles and for the amounts of haemoglobin in the blood. Color indices are also given. Basophiles were absent in some males and females of all age groups. Eosinophiles were not distinguished in a number of males and females under two months of age.

In a previous paper (2) the materials and methods used for examining the blood of normal mink were described and a description of the cellular morphology of mink blood was given.

In this paper the numbers of the various types of cells are recorded in Tables I to X. The number of grams of haemoglobin per cubic millimetre (Hb. gm. per cu. mm.) was estimated by the Sahli haemoglobinometer and the haemoglobin index determined by dividing the number of grams of haemoglobin in 100 cc. of blood by the number (in millions) of red blood cells in one cubic millimetre. The formula for calculating the color index is that given by Cullen (1).

The mink are grouped according to age, ranging from under two months of age to adult mink. The red blood corpuscles (R.b.cs.) are estimated to the nearest thousand per cu. mm. of blood and for purposes of tabulation are stated in millions. The total numbers of white blood corpuscles (W.b.cs.) per cu. mm. are recorded without reductions from the actual count. The lymphocyte (Ly.), monocyte (Mo.), neutrophile (Ne.), eosinophile (Eo.) and basophile (Ba.) leucocytes occurring in a cu. mm. of blood are expressed as percentages of the total and also as round numbers.

The numerical range covered by the various groups has been indicated at the foot of each column by tabulating the minimum, maximum and mean figures. The standard deviation has been calculated by dividing the sum of the squares of the deviations from the mean by the number of individual cases recorded, and finding the square root of the result. The standard deviation has been calculated to give all the deviations their true value. In order to have the amounts of variation expressed on a common basis for comparison the coefficient of variation is used. This is the standard deviation expressed as a percentage of the mean.

[1] *Original manuscript received November 30, 1934.*
Contribution from the Ontario Government Experimental Fur Farm, Kirkfield, Ontario.
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Numbers of Blood Elements in Normal Mink

The Red Blood Corpuscles

The average numbers of red blood corpuscles per cubic millimetre of mink blood for all the groups examined were—for males, 7,941,000; for females, 8,133,000. The maximum number for males, 12,288,000, occurred in a mink between five and seven months old. The maximum number for females was 12,360,000 which occurred in a female also between five and seven months of age. The minimum number for all the male groups of mink was 3,704,000 which occurred in a mink kitten 42 days old. The minimum number for all the female groups was 3,584,000 which occurred in a mink kitten 54 days old.

As the age of the mink increased the average number of red blood cells for each group also increased. The greatest differences in individual animals occurred in both males and females between five to seven months old. In these groups the maximum number per cu. mm. of blood for males was 12,288,000 and for females 12,264,000; the minimum number for males was 7,816,000, and for females 7,808,000. Smaller differences in numbers of red blood cells occurred in the adults than in any of the other groups of mink.

TABLE I

NUMBERS OF THE BLOOD ELEMENTS IN MINK UNDER TWO MONTHS OF AGE

Males				Females			
No.	R.b.cs.	Hb. gm.	Ind.	No.	R.b.cs.	Hb. gm.	Ind.
1	5.392	7.9	0.73	1	6.648	8.5	0.40
2	5.528	7.6	0.42	2	6.256	7.7	0.43
3	5.304	8.5	0.57	3	6.872	8.1	0.40
4	5.432	8.2	0.55	4	5.560	7.6	0.42
5	4.808	8.0	0.53	5	4.936	7.8	0.52
6	4.696	8.6	0.57	6	5.536	7.8	0.43
7	6.024	9.0	0.50	7	5.672	8.0	0.44
8	3.704	4.7	0.52	8	5.608	7.5	0.42
9	4.776	5.3	0.44	9	5.792	7.7	0.43
10	5.280	7.7	0.51	10	3.584	6.9	0.57
11	5.880	8.5	0.47	11	5.064	7.2	0.48
12	5.800	8.3	0.46	12	6.160	8.5	0.47
13	6.640	7.0	0.33	—	—	—	—
Min.	3.704	4.7	0.33	Min.	3.584	6.9	0.40
Max.	6.640	9.0	0.57	Max.	6.872	8.5	0.57
Mean	5.324	7.6	0.49	Mean	5.641	7.8	0.45
S.D.	±1.294	±1.2	±0.12	S.D.	±0.819	±0.45	±0.05
C. of V.	26	16	23	C. of V.	15	6	11

Grams of Haemoglobin per 100 cc. of Blood

The average numbers of grams of haemoglobin per 100 cc. of blood for all the groups examined were, for males 10.8, for females 11.8, with a maximum of 17.7 for males and 17.3 for females. These amounts occurred in males

between five and seven months of age and in adult females. The minimum for males (4.7) and for females (6.9), occurred in individuals under two months of age.

TABLE II

NUMBERS OF THE BLOOD ELEMENTS IN MINK UNDER TWO MONTHS OF AGE

Males							Females						
No.	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.	No.	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.
1	6000	74.5	0.0	22.5	2.5	0.5	1	5200	62.5	2.5	34.5	0.5	0.0
		4470	000	1350	150	30			3250	130	1794	26	000
2	6200	44.0	2.0	49.0	4.0	1.0	2	5200	55.5	2.0	42.0	0.0	0.5
		2728	124	3038	248	62			2886	104	2184	000	26
3	8000	64.5	1.0	33.5	1.0	0.0	3	5400	56.0	1.5	38.5	3.0	1.0
		5160	80	2680	80	000			3024	81	2079	162	54
4	8000	67.5	1.0	29.5	1.5	0.5	4	5600	62.5	0.5	35.0	1.5	0.5
		5400	80	2360	120	40			3500	28	1960	84	28
5	4200	74.5	0.0	25.0	0.0	0.5	5	10800	61.5	0.0	38.0	0.0	0.5
		3129	000	1050	000	21			6642	000	4104	000	54
6	7600	66.5	3.5	27.5	1.0	1.5	6	7200	60.5	0.0	38.5	0.5	0.5
		5054	266	2090	76	114			4356	000	2772	36	36
7	7200	71.5	3.0	24.0	0.0	1.5	7	10000	56.5	0.0	43.5	0.0	0.0
		5148	216	1728	000	108			5650	000	4350	000	000
8	9400	62.0	0.5	37.5	0.0	0.0	8	9200	77.5	1.0	19.0	1.5	1.0
		5828	47	3525	000	000			7130	92	1748	138	92
9	8600	63.0	1.0	34.5	0.5	1.0	9	6600	84.5	0.5	15.0	0.0	0.0
		5418	86	2967	43	86			5577	33	990	000	000
10	6600	67.0	1.5	31.5	0.0	0.0	10	7200	78.0	0.0	22.0	0.0	0.0
		4422	99	2079	000	000			5616	000	1584	000	000
11	5800	70.0	2.0	27.5	0.0	0.5	11	7600	62.5	2.0	35.0	0.5	0.0
		4060	116	1595	000	29			4750	152	2660	38	000
12	6600	59.0	0.5	39.5	1.0	0.0	12	9400	88.0	0.5	11.0	0.5	0.0
		3894	33	2607	66	000			8272	47	1034	47	000
13	6600	72.0	1.5	26.0	0.5	0.0	—	—	—	—	—	—	—
		4752	99	1716	33	000			—	—	—	—	—
Min.	4200	44.0	0.0	22.5	0.0	0.0	Min.	5200	55.5	0.0	11.0	0.0	0.0
Max.	9400	74.5	3.5	49.0	4.0	1.5	Max.	10800	88.0	2.5	42.0	3.0	1.0
Mean	7000	65.8	1.3	31.3	0.9	0.5	Mean	7450	67.1	0.9	31.0	0.7	0.3
S.D.	±1304	±7.7	±1.0	±7.1	±1.1	±0.5	S.D.	±1893	±11.09	±0.86	±10.6	±0.89	±0.37
C. of V.	19	12	77	23	122	100	C. of V.	25	17	96	34	127	123
Min.		2728	000	1050	000	000	Min.		2886	000	990	000	000
Max.		5828	266	3525	248	114	Max.		7130	152	4350	162	92
Mean		4574	96	2214	63	38	Mean		5054	56	2272	44	24
S.D.		±884	±73	±699	±72	±40	S.D.		±1658	±51	±1015	±52	±29
C. of V.		19	76	31	114	105	C. of V.		33	91	45	118	121

The amounts of haemoglobin increased with age in the female groups which show an average of 15.0 gm. in the adult group. In the male groups the amounts increased up to seven months of age when the average for the group was 14.3 gm. In the adult males there was a decrease, the average for the group being 11.9 gm.

Color Index

For the color index, the haemoglobin content of the red blood cells was calculated as gm. haemoglobin in 100 cc. of blood per one million red blood cells in one cubic millimetre.

The averages for all groups examined were .46 for males and .48 for females. Extreme limits for males were .76 to .33; for females .64 to .39. The highest index reached in male mink occurred between two and four months of age, and in female mink between five and seven months of age. The lowest index reached in male mink was in an individual under two months old and the lowest in female mink in an animal between two and four months old.

The female adult mink showed a higher color index than the adult males. The averages were:—adult female group .52, adult male group .41.

TABLE III

NUMBERS OF THE BLOOD ELEMENTS IN MINK OVER TWO AND UNDER FIVE MONTHS OF AGE

Males				Females			
No.	R.b.cs.	Hb. gm.	Ind.	No.	R.b.cs.	Hb. gm.	Ind.
1	7.840	10.2	0.42	1	7.992	11.5	0.48
2	7.208	9.4	0.45	2	9.200	10.5	0.39
3	7.712	10.6	0.44	3	8.744	10.4	0.38
4	4.200	9.1	0.76	4	8.464	10.2	0.42
5	6.560	9.8	0.47	5	7.784	9.9	0.41
6	6.896	9.4	0.45	6	7.752	9.3	0.39
7	7.472	10.5	0.50	7	7.528	9.9	0.41
8	6.176	9.5	0.53	8	6.752	9.9	0.47
9	7.768	8.9	0.37	9	5.536	9.7	0.54
10	8.008	9.5	0.40	10	7.360	9.9	0.47
11	8.552	9.5	0.35	11	5.432	8.9	0.59
12	8.112	9.0	0.37	12	7.912	10.5	0.44
13	6.720	9.5	0.45	13	7.096	9.5	0.45
Min.	4.200	8.9	0.35	Min.	5.432	8.9	0.38
Max.	8.552	10.6	0.76	Max.	9.200	11.5	0.59
Mean	7.171	9.6	0.46	Mean	7.489	10.0	0.45
S.D.	±1.051	±0.51	±0.01	S.D.	±0.967	±0.6	±0.06
C. of V.	15	5	2	C. of V.	13	6	13

White Blood Corpuscles

The average number of white blood corpuscles for all male groups examined was 7,902. A maximum of 16,400 occurred in a mink between five and seven months of age and a minimum of 3,800 in an adult mink. The average

number of white blood cells found in the female mink was 8,047. A maximum of 15,000 occurred in a mink between five and seven months of age. A minimum of 4,600 occurred in a mink between five and seven months of age.

TABLE IV

NUMBERS OF THE BLOOD ELEMENTS IN MINK OVER TWO AND UNDER FIVE MONTHS OF AGE

Males							Females						
No.	W.b.ca.	Ly.	Mo.	Ne.	Eo.	Ba.	No.	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.
1	8200	48.0 3936	0.5 41	47.5 3895	4.0 328	0.0 000	1	8200	55.0 4510	0.0 000	29.5 2419	15.5 1271	0.0 000
2	15400	54.5 8393	0.5 77	28.0 4312	16.5 2541	0.5 77	2	10400	73.0 7592	0.5 52	19.0 1976	7.0 728	0.5 52
3	7400	44.5 3293	0.5 37	54.0 3996	1.0 74	0.0 000	3	7600	50.0 3800	2.0 152	36.5 2774	11.0 836	0.5 38
4	11200	33.0 3696	1.0 112	64.5 7224	1.0 112	0.5 56	4	10600	59.5 6307	1.0 106	35.0 3710	4.0 424	0.5 53
5	12400	25.0 3100	1.0 124	70.0 8680	2.0 248	2.0 248	5	6800	57.5 3910	0.0 000	39.0 2652	3.5 238	0.0 000
6	6800	31.0 2108	5.0 340	53.0 3604	9.5 646	1.5 102	6	12200	55.5 6771	0.0 000	40.0 4880	4.5 549	0.0 000
7	8400	33.5 2814	1.5 126	60 0 5040	3.5 294	1.5 126	7	7600	71.0 5434	1.0 76	25.5 1938	1.5 114	0.5 38
8	6800	54.0 3672	0.5 34	39.0 2652	6.0 408	0.5 34	8	11000	49.5 5445	1.5 165	46.5 5115	1.5 165	1.0 110
9	7200	37.5 2700	2.5 180	58.5 4212	1.5 108	0.0 000	9	12800	43.5 5568	0.0 000	45.5 5824	10.5 1344	0.5 64
10	6800	32.0 2176	3.0 204	52.0 3536	10.0 680	3.0 204	10	8600	29.5 2537	1.5 129	67.0 5762	2.0 172	0.0 000
11	5000	49.0 2450	1.0 50	41.0 2050	7.0 350	2.0 100	11	9800	51.5 5047	2.5 245	44.5 4361	1.0 98	0.5 49
12	10400	25.0 2600	3.0 312	70.5 7332	1.0 104	0.5 52	12	5600	36.5 2044	4.0 224	51.0 2856	7.0 392	1.5 84
13	9200	41.0 3772	3.0 276	47.5 4370	8.5 782	0.0 000	13	5800	37.5 2175	2.0 116	53.5 3103	7.0 406	0.0 000
Min.	5000	25.0	0.5	28.0	1.0	0.0	Min.	5600	29.5	0.0	19.0	1.0	0.0
Max.	15400	54.5	5.0	70.5	16.5	3.0	Max.	12800	73.0	4.0	67.0	15.5	1.5
Mean	8862	39.0	1.8	52.7	5.5	0.9	Mean	9000	52.0	1.2	41.0	5.8	0.4
S.D.	±2719	±9.8	±1.3	±11.8	±4.5	±0.9	S.D.	±2285	±12.3	±1.1	±12.1	±4.2	±0.4
C. of V.	31	25	72	22	82	100		25	24	92	29	72	100
Min.		2108	34	2050	74	000	Min.		2044	000	1938	98	000
Max.		8393	340	8680	2541	248	Max.		7592	245	5824	1271	110
Mean		3439	147	4685	513	77	Mean		4703	97	3644	518	38
S.D.		±1548	±32	±1853	±627	±76	S.D.		±1592	±82	±1339	±401	±35
C. of V.		45	22	40	122	99	C. of V.		34	85	37	77	92

The average number of white blood cells showed a steady increase in the young mink, with greater differences between individual animals as the groups increased in age. The white blood cells decreased in numbers in adult mink, the average number for the males being 6,380 and for females 7,800.

Lymphocytes

For all male groups the lymphocytes averaged 3,957 (49.0%) cells per cu. mm. of blood and for the female groups the average was 4,269 (53.1%). The maximum number of lymphocytes in the male groups was 11,562 (70.5%)

TABLE V

NUMBERS OF THE BLOOD ELEMENTS IN MINK OVER FIVE AND UNDER SEVEN MONTHS OF AGE

Males				Females			
No.	R.b.cs.	Hb. gm.	Ind.	No.	R.b.cs.	Hb. gm.	Ind.
1	7.816	10.0	0.41	1	7.808	11.3	0.47
2	8.832	11.5	0.42	2	9.952	13.0	0.43
3	8.976	13.3	0.49	3	8.752	13.0	0.48
4	8.968	13.5	0.50	4	7.880	12.8	0.53
5	9.584	15.4	0.51	5	9.472	17.2	0.64
6	11.056	17.7	0.54	6	9.912	15.4	0.51
7	12.288	16.7	0.46	7	12.176	15.0	0.42
8	9.928	16.7	0.56	8	12.264	15.8	0.44
9	9.072	16.0	0.59	9	9.288	14.9	0.55
10	8.960	12.4	0.46	10	9.288	16.0	0.59
Min.	7.816	10.0	0.41	Min.	7.808	11.3	0.42
Max.	12.288	17.7	0.59	Max.	12.264	17.2	0.64
Mean	9.548	14.3	0.49	Mean	9.679	14.4	0.51
S.D.	±1.212	±2.42	±0.06	S.D.	±1.344	±1.7	±0.06
C. of V.	13	17	12	C. of V.	14	12	12

found in a mink between five and seven months old, and the minimum was 900 (22.5%). The latter occurred in the adult male group. The maximum number for the female groups was 10,875 (72.5%) found in a mink between five and seven months old and the minimum was 1,470 (21.0%) also in a mink between five and seven months old. The greatest differences occurred in the groups between five and seven months of age. Greater differences occurred among female mink than among males. Male and female mink under two months of age showed a higher proportion of lymphocytes in the blood than those of any of the other age groups. The maximum proportion of lymphocytes in males, 74.5%, occurred in two individuals; the minimum proportion was 44.0% and the average proportion for the group 65.8%. The maximum proportion of lymphocytes in female mink of the same age was 88.0%; the minimum proportion was 55.5% and the average proportion for the group 67.1%.

Monocytes

The average number of monocytes occurring in the blood of male mink for all groups examined was 157 (1.8%) with extreme limits of 627 (5.5%)

to 0, the last in a mink between five and seven months of age. The average for females was 90 (1.2%) with limits of 420 (5.0%) to 0 occurring in the adults. The greatest number of monocytes was found in mink between five and seven months of age. The smallest number of monocytes was found in mink under two months of age. The greatest fluctuations among the individual animals occurred in the adult groups. The smallest fluctuations were found in the mink under two months of age.

TABLE VI

NUMBERS OF THE BLOOD ELEMENTS IN MINK OVER FIVE AND UNDER SEVEN MONTHS OF AGE

Males							Females						
No.	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.	No.	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.
1	8000	62.5	2.5	28.0	7.0	0.0	1	13200	32.0	1.0	65.0	2.0	0.0
		5000	200	2240	560	000			4224	132	8580	264	000
2	11400	49.0	5.5	30.5	14.5	0.5	2	6000	36.5	1.5	52.5	9.0	0.5
		5586	627	3477	1653	57			2190	90	3150	540	30
3	8000	43.0	3.5	44.5	8.0	1.0	3	9600	66.0	3.0	28.5	2.0	0.5
		3440	280	3560	640	80			6336	288	2736	192	48
4	10000	53.5	1.5	40.0	4.5	0.5	4	15000	72.5	1.0	19.0	7.5	0.0
		5350	150	4000	450	50			10875	150	2850	1125	000
5	13200	60.5	3.0	29.0	7.5	0.0	5	6000	49.5	1.0	36.0	13.5	0.0
		7986	396	3828	990	000			2970	60	2160	810	000
6	16400	70.5	2.0	19.0	8.5	0.0	6	6400	27.0	2.5	64.0	6.5	0.0
		11562	328	3116	1394	000			1728	160	4096	416	000
7	10600	57.5	2.5	36.5	3.0	0.5	7	5400	54.5	0.5	39.5	4.0	1.5
		6095	265	3869	318	53			2943	27	2133	216	81
8	5800	29.5	5.0	57.5	8.0	0.0	8	7000	21.0	0.0	74.5	4.5	0.0
		1711	290	3335	464	0.0			1470	000	5215	315	000
9	5400	31.5	3.5	54.5	10.0	0.5	9	6200	35.0	0.0	65.0	0.0	0.0
		1701	189	2943	540	27			2170	000	4030	000	000
10	5000	19.5	0.0	76.5	4.0	0.0	10	4600	63.0	0.5	32.5	4.0	0.0
		975	000	3825	200	000			2898	23	1495	184	000
Min.	5000	19.5	0.0	19.0	3.0	0.0	Min.	4600	21.0	0.0	19.0	0.0	0.0
Max.	16400	70.5	5.5	76.5	14.5	1.0	Max.	15000	72.5	3.0	74.5	13.5	1.5
Mean	9380	47.70	2.90	41.60	7.50	.30	Mean	7940	45.70	1.10	47.70	5.30	.25
S.D.	±3485	±15.64	±1.53	±12.99	±3.14	±0.40	S.D.	±3189	±16.9	±0.81	±17.7	±3.8	±0.45
C. of V.	37	33	53	31	42	133	C. of V.	40	37	74	37	72	140
Min.		975	150	2240	200	000	Min.		1470	000	1495	000	000
Max.		11562	627	4000	1653	80	Max.		10875	288	8580	1125	81
Mean		4941	273	3419	721	27	Mean		3780	93	3645	406	16
S.D.		±3045	±164	±510	±440	±29	S.D.		±2718	±87	±1949	±320	±27
C. of V.		62	60	15	61	108	C. of V.		72	94	53	79	169

Neutrophiles

The average number of neutrophiles for the male mink groups examined was 3,299 (42.3%); and that for the female groups was 3,311 (41.5%). The extreme limits for the males were 8,680 (70.0%) and 1,050 (25.0%). Limits for the females were 8,580 (65.0%) and 1,034 (11.0%). The lowest proportion of neutrophiles for the individual animals was found in the mink under two months of age. There is a slight increase in the average numbers of

TABLE VII

NUMBERS OF THE BLOOD ELEMENTS IN ADULT MINK

Males				Females			
No.	R.b.cs.	Hb. gm.	Ind.	No.	R.b.cs.	Hb. gm.	Ind.
1	9.512	10.2	0.34	1	8.336	11.7	0.49
2	9.176	9.9	0.37	2	7.504	11.4	0.48
3	9.800	9.9	0.33	3	8.624	16.0	0.59
4	10.352	10.7	0.36	4	11.320	17.0	0.51
5	9.872	9.5	0.32	5	9.104	16.0	0.59
6	8.952	12.5	0.46	6	11.040	17.3	0.52
7	9.016	12.9	0.48	7	10.784	15.1	0.46
8	9.552	13.6	0.45	8	10.248	16.1	0.54
9	10.424	14.4	0.48	9	9.544	14.5	0.48
10	10.176	15.6	0.52	—	—	—	—
Min.	8.952	9.5	0.32	Min.	7.504	11.4	0.46
Max.	10.424	15.6	0.52	Max.	11.320	17.3	0.59
Mean	9.683	11.9	0.41	Mean	9.723	15.0	0.52
S.D.	±0.507	±2.06	±0.07	S.D.	±1.254	±2.0	±0.04
C. of V.	5	17	17	C. of V.	13	13	8

neutrophiles per cu. mm. of blood from the young female to the adult female mink. In the group under two months of age the number was 2,272 (31.0%). In adults the number was 3,683 (46.3%). The highest average number for the male groups was 4,685 (52.7%), found in males between two and four months of age. The lowest number, 2,214 (31.3%), was found in the male group under two months of age. In the adult male group the average number decreased to 2,880 (47.1%).

Eosinophiles

The average number of eosinophiles per cu. mm. of blood for all groups examined was: males 438 (5.3%), with extreme limits of 2,541 (16.5%) to 0; females 314 (3.9%), with extreme limits of 1,344 (10.5%) to 0. The highest average number of eosinophiles for all the male groups was in the group five to seven months of age, where 721 (7.5%) per cu. mm. of blood was found. The lowest average number for the male groups, 63 (0.9%), was found in the group under two months of age. The highest average number for the female groups was found in the group two to four months of age which showed 518 (5.8%) per cu. mm. of blood. The lowest average number

for all the female groups, 44 (0.7%), was found in the group under two months of age. In a number of the animals in both male and female groups of mink under two months of age the presence of eosinophiles was not established.

Basophiles

The average number of basophiles occurring in the blood of male mink for all the groups examined was 49 (0.6%), with extreme limits of 248 (2.0%)

TABLE VIII

NUMBERS OF THE BLOOD ELEMENTS IN ADULT MINK

Males							Females						
No.	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.	No.	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.
1	7400	46.0 3404	1.5 111	45.0 3330	6.5 481	1.0 74	1	8600	50.5 4343	4.5 387	41.0 3526	3.0 258	1.0 86
2	10200	47.5 4845	4.0 408	42.5 4335	5.5 561	0.5 51	2	8400	30.0 2520	5.0 420	51.0 4284	13.0 1092	1.0 84
3	4800	47.0 2256	1.0 48	48.5 2328	2.5 120	1.0 48	3	8800	39.5 3476	0.5 44	58.5 5148	0.0 000	1.5 132
4	5000	50.5 2525	1.0 50	42.0 2100	5.5 275	1.0 50	4	7000	68.5 4795	0.0 000	26.5 1855	4.0 280	1.0 70
5	9600	46.0 4416	0.0 000	50.0 4800	3.0 288	1.0 96	5	6200	67.0 4154	0.0 000	29.0 1798	4.0 248	0.0 000
6	6000	57.5 3450	1.5 90	25.0 1500	15.5 930	0.5 30	6	7200	44.0 3168	0.5 36	51.0 3672	3.0 216	1.5 108
7	7200	57.5 4140	5.5 396	18.5 1332	16.0 1152	2.5 180	7	12200	33.5 3752	0.5 56	65.5 7336	0.0 000	0.5 56
8	3800	33.5 1273	0.0 000	63.5 2413	3.0 114	0.0 000	8	6600	55.0 3630	0.0 000	44.0 2904	0.5 33	0.5 33
9	5800	26.5 1537	0.0 000	69.0 4002	4.5 261	0.0 000	9	5200	39.0 2028	2.0 104	50.5 2626	8.5 442	0.0 000
10	4000	22.5 900	1.0 40	66.5 2660	9.5 380	0.5 20	—	—	— —	— —	— —	— —	— —
Min.	3800	22.5	0.0	18.5	2.5	0.0	Min.	5200	30.0	0.0	26.5	0.0	0.0
Max.	10200	57.5	5.5	69.0	16.0	2.5	Max.	12200	68.5	5.0	65.5	13.0	1.5
Mean	6380	43.5	1.1	47.1	7.2	0.8	Mean	7800	47.5	1.5	46.3	4.0	0.8
S.D.	±2095	±11.5	±1.8	±15.8	±4.7	±0.68	S.D.	±1496	±12.1	±1.9	±12.0	±4.0	±0.54
C. of V.	33	26	164	34	65	85	C. of V.	19	25	127	26	100	66
Min.		900	000	1332	114	000	Min.		2028	000	1798	000	000
Max.		4845	408	4800	1152	180	Max.		4795	420	7336	1092	132
Mean		2875	114	2880	456	55	Mean		3541	116	3683	285	63
S.D.		±1312	±149	±1129	±325	±51	S.D.		±831	±157	±1675	±318	±42
C. of V.		46	130	39	71	93	C. of V.		23	135	45	112	67

to 0 occurring in mink between two and five months of age. The average for females was 35 (0.4%) with limits of 132 (1.5%) to 0 occurring in adult mink. The highest average number of basophiles for the male group, 77 (0.9%) was found in the group from two to five months of age. Among the females the highest average number, 63 (0.8%), was found in the adult

TABLE IX

FINAL MEANS OF THE BLOOD ELEMENTS IN ALL GROUPS OF MINK

Males				Females			
Age, months	R.b.cs.	Hb. gm.	Ind.	Age, months	R.b.cs.	Hb. gm.	Ind.
0–2	5.324	7.6	0.49	0–2	5.641	7.8	0.45
2–3	7.171	9.6	0.46	2–3	7.489	10.0	0.45
5–7	9.548	14.3	0.49	5–7	9.679	14.4	0.51
Yr. 1–3	9.683	11.9	0.41	Yr. 1–3	9.723	15.0	0.52
Min.	5.324	7.6	0.41	Min.	5.641	7.8	0.45
Max.	9.683	14.3	0.49	Max.	9.723	15.0	0.52
Mean	7.941	10.8	0.46	Mean	8.133	11.8	0.48
S.D.	±1.723	±3.0	±0.03	S.D.	±1.918	±2.5	±0.04
C. of V.	22	27	6	C. of V.	24	21	8

TABLE X

FINAL MEANS OF THE BLOOD ELEMENTS IN ALL GROUPS OF MINK

Males							Females						
Age, months	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.	Age, months	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.
0–2	6985	65.8	1.3	31.3	0.9	0.5	0–2	7450	67.1	0.9	31.0	0.7	0.3
		4574	96	2214	63	38			5054	56	2272	44	24
2–3	8862	39.0	1.8	52.7	5.5	0.9	2–3	9000	52.0	1.2	41.0	5.8	0.4
		3439	147	4685	513	77			4703	97	3644	518	38
5–7	9380	47.7	2.9	41.6	7.5	0.3	5–7	7940	45.7	1.1	47.7	5.3	0.2
		4941	273	3419	721	27			3780	93	3645	406	16
Yr. 1–3	6380	43.5	1.1	47.1	7.2	0.8	Yr. 1–3	7800	47.5	1.5	46.3	4.0	0.8
		2875	114	2880	456	55			3541	116	3683	285	63
Min.	6380	39.0	1.1	31.3	0.9	0.3	Min.	7450	45.7	0.9	31.0	0.7	0.2
Max.	9380	65.8	2.9	52.7	7.5	0.9	Max.	9000	67.1	1.5	47.7	5.8	0.8
Mean	7902	49.0	1.8	43.2	5.3	0.6	Mean	8047	53.1	1.2	41.5	3.9	0.4
S.D.	±578	±8.4	±0.7	±6.0	±2.6	±0.2	S.D.	±1251	±10.2	±0.7	±7.9	±2.6	±0.2
C. of V.	7	17	39	14	49	33	C. of V.	16	19	58	19	67	50
Min.		2875	96	2214	63	38	Min.		3541	56	2272	44	16
Max.		4941	273	4685	721	77	Max.		5054	116	3683	518	63
Mean		3957	157	3299	438	49	Mean		4269	90	3311	314	35
S.D.		±627	±22	±600	±176	±18	S.D.		±835	±69	±907	±238	±18
C. of V.		16	14	18	40	37	C. of V.		20	77	27	76	51

group. The lowest average number for all the male groups, 38 (0.5%), was found in the group under two months of age. The lowest average number for the female groups, 16 (0.2%) was found in the group between five and seven months of age. In all the groups of both the male and female mink, a number of individual animals did not reveal the presence of basophiles in their blood.

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CYTOLOGY OF THE BLOOD OF NORMAL MINK AND RACCOON

III. MORPHOLOGY AND NUMBERS OF THE BLOOD ELEMENTS IN RACCOON[1]

By Arnold H. Kennedy[2]

Abstract

Maximum, minimum and average values for numbers and dimensions of the elements in the blood of normal raccoon are given. Normoblasts are rare and anisocytosis was found only in animals from one to three months old. Lymphocytes could not be classified into size groups as they completely cover a wide range from small to large. In the monocytes the nuclei are prominent and boldly outlined but vary in shape. In the neutrophiles the nuclei have from two to seven segments, five being most common. The eosinophiles are large and contain oval, S-shaped, or lobed nuclei. The nuclei of the basophiles are usually bar- or S-shaped, seldom lobed. Two types of basophilic cells may be distinguished. One contains 8–40 prominent granules while the other contains a mass of granular material with occasionally a few large granules. In addition to descriptions of these elements, data are supplied on haemoglobin content and color index of raccoon blood.

The methods used for examining the raccoon blood were those described in previous papers (1, 2).

Blood examinations made on raccoon during the hibernating season are not being considered at this time. The raccoon were grouped according to age, ranging from under three months to adults. The cellular morphology and the numbers of the blood elements for raccoon are considered here.

Morphology of Raccoon Blood

The red blood corpuscles take up the eosin from Hasting's stain, giving a slight orange red to dark red color. The staining is diffused evenly throughout the cell and no fine structures are visible. The centre appears paler than the periphery, owing to the biconcave formation of the corpuscle.

The average diameter of the red blood corpuscle is 6.5 μ with extreme limits of 5.0 to 9.0 μ.

Normoblasts were very rare in raccoon blood, only one or two being observed in the examination of all the films from the various age groups.

Anisocytosis was not found to any extent except in raccoon from one to three months old.

Lymphocytes (Plate I, Figs. 1 and 2)

The lymphocytes of the raccoon appear very much like the lymphocytes of the mink (1). Two forms are found, one large, the other small. Intermediate forms occur, making it impossible to separate the lymphocytes into two distinct groups. The small forms are by far the most numerous. In the larger forms the nucleus may be rounded, bean-shaped or irregular in outline. It may be situated near the margin of the cell or more centrally located, and stains a light purple color with the nuclear material appearing

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Contribution from the Ontario Government Experimental Fur Farm, Kirkfield, Ontario.
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more uniform and granular in appearance than in the smaller forms. The nuclei of the larger lymphocytes are surrounded by a greater amount of pale blue staining cytoplasm than are the nuclei of the smaller forms.

The small lymphocytes have a small, compact, round or oval nucleus, with the nuclear masses staining a deep purple color. The heavier staining nuclear masses are separated by clear open spaces which give the nuclei an uneven, slightly wavy appearance. The nucleus may be surrounded by a light-blue staining cytoplasm, which in some cells can hardly be discerned owing to the light staining, or by a very narrow margin of cytoplasm, but a narrow ring or crescent on one side is the most common form.

The lymphocytic cytoplasm may contain from three to sixteen reddish-brown granules. These may easily be discerned whenever they occur.

The largest lymphocyte observed was 19 0 by 12.0 μ, with the nuclei 12.0 by 12.0 μ. The average size was 11.0 by 9.4 μ with the nuclei 10.7 by 8.2 μ. The minimum size was 6.0 by 5.0 μ, with the nuclei 5.0 by 5.0 μ.

Monocytes (Plate I, Figs. 3 and 4)

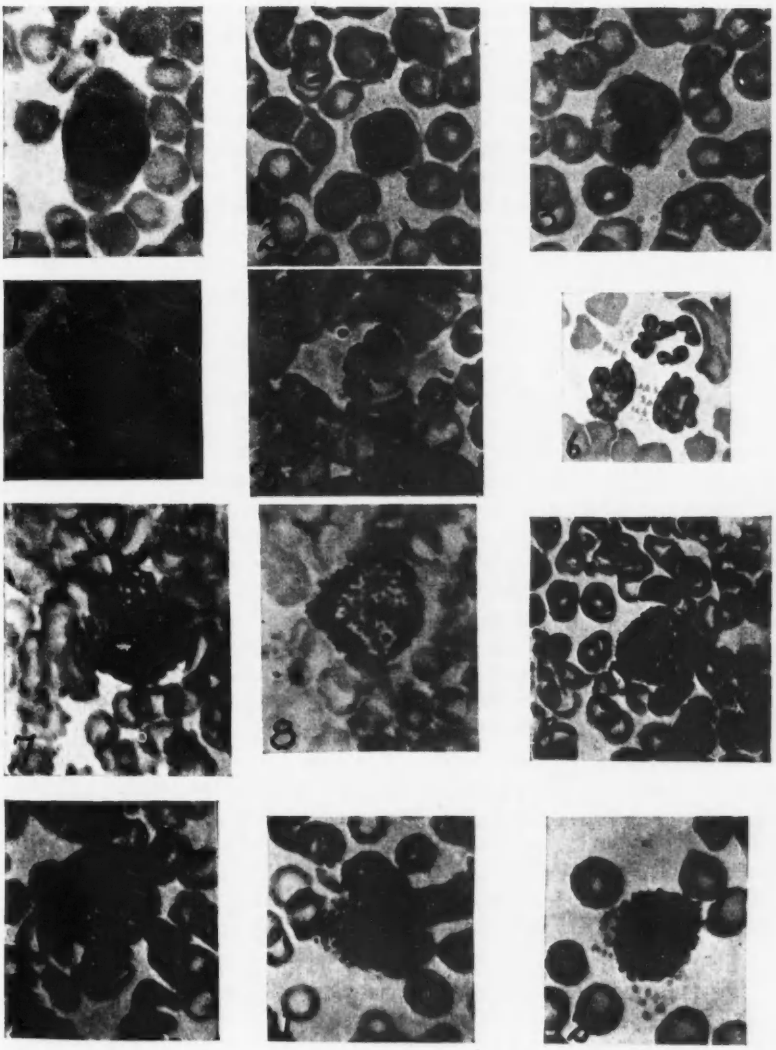
The cytoplasm of the monocytes stains a light blue or faint greenish-blue color. No granules were observed. The nuclei are prominent, of various shapes and boldy outlined. Kidney-, band-, horseshoe-, and L-shaped forms were most common. In the smaller monocytes the nucleus appeared more rounded, thicker, and oval. Occasionally a nucleus with three lobes would be observed. These occurred more often in the larger monocytes. The nucleus, staining from a light to a reddish purple, is usually located near the outer margin of the cell with the convex side towards the margin. The more rounded nuclei are often more centrally located. The maximum size of the monocyte is 18.0 by 12.0 μ and the average size 14.8 by 10.8 μ; the size of the forms most commonly found lies between 12.0 and 14.0 μ. The minimum size is 10.3 by 9.0 μ.

Neutrophiles (Plate I, Figs. 5 and 6)

The cytoplasm of the neutrophile stains a very light, sky-blue color. A number of small, faintly stained, reddish-blue granules may be discerned throughout the cytoplasm, especially in young raccoons' blood. The granules stain so faintly in many cells that their presence may often be unnoticed. The nuclei stain from deep blue to purple, are segmented and sharply divided into bands by the oxyphile portion of the nuclear material. The numbers of segments vary from two to seven, five being the most common. The segments are connected by fine, very distinct filaments of chromatin. Band-form nuclei are observed, especially in the younger groups of raccoon, and constitute from two to seven per cent of the total number of neutrophiles.

Metamyelocytes are also observed and approximate from 0.5 to 2.0% of the total number of neutrophiles. Occasionally a myelocyte may be observed but they appear to be very rare in raccoon blood.

The maximum size of the neutrophiles is 16.0 by 13.0 μ; the average size is 12.8 by 11.0 μ and the minimum size is 10.2 by 8.2 μ.



FIGS. 1 and 2. *Lymphocytes.* FIGS. 3 and 4. *Monocytes.* FIGS. 5 and 6. *Neutrophiles.* FIGS. 7, 8 and 9. *Eosinophiles.* FIGS. 10, 11 and 12. *Basophiles.* × *14.*

Eosinophiles (Plate I, Figs. 7, 8 and 9)

The eosinophile in raccoon blood is large, prominent and bold in outline, with a tendency to have slightly frayed or roughened borders. The granules stain bright red to pink. They are numerous, large, and prominent, and fill up the cell, often projecting over the borders, and they may completely obscure the cytoplasm and portions of the nucleus. The nucleus is very simple, frequently oval, S-shaped, or in the form of a curved bar or divided into two or three lobes, which in all probability are joined by chromatin threads, but the threads are always obscured by the granules. The nuclei stain from a sky-blue to a deep blue or purplish color. The chromatin usually has a uniform appearance. In broken down cells the granules can be seen more clearly. They appear round to oval in shape and measure approximately 0.5 to 0.7 μ.

The maximum size of the eosinophile is 18.0 by 13.0 μ; the average size is 13.0 by 10.4 μ and the minimum 11.0 by 6.0 μ.

Basophiles (Plate I, Figs. 10, 11 and 12)

The nuclei of the basophile cells are usually quite prominent and not obscured to any extent by granules. They are usually band-, bar-, or S-shaped and are very seldom divided or lobed. They stain a light purple color and are divided into bands by the oxyphile portion of the nuclear material, which takes a color similar to that taken by the cytoplasm of the cell. In staining reaction and appearance, they are very similar to band-formed neutrophiles. The basophiles, in raccoon blood, contain two types of granules; these types occurring in different cells. The greater number of basophile cells contain numerous granules which often fill the cell. These stain a very faint light-blue to a faint smoky-blue color. They are about the size of the granules of the eosinophile cells or a little smaller, but are less prominently defined in outline. Because of their faint, indistinct appearance these smaller granules may often be overlooked, and the cell classified as a neutrophile. A few large deep-purple granules may be present in the cytoplasm of these cells. Basophilic cells are also found which contain not the smaller, more faintly staining granules which fill the cytoplasm, but prominent large sized granules, 8 to 40 in number, measuring around 1.0 μ and staining a bright purple color. These granules may appear over the nucleus and they also appear on the outer surface of the cytoplasm (Plate I, Fig. 12). The cytoplasm of these cells stains lilac or light mauve. The number of basophiles in the raccoon is too low to give any accurate indication of the proportions of the two types of cells.

Both types of basophilic cells are of approximately the same size. The maximum size is 15.0 by 11.0 μ, the average 13.2 by 10.5 μ and the minimum 11.0 by 9.0 μ.

Degenerate Forms of Cells Found in Raccoon Blood

Under this heading are placed those cells that have lost their characteristic identity and possess no definite structure. The majority of them most

probably are lymphocytic and monocytic in origin. They stain greenish blue to dark blue and often have a faded and frayed appearance.

Platelets

Platelets often appeared in large masses and their individual identity could not be discerned. Where they appeared singly they were round, oblong or irregular in outline. The protoplasm stained a pale, sky-blue color and contained clumps or rings of dark-blue-staining structures throughout the cytoplasm.

The maximum size for platelets in raccoon blood is 5.0 by 3.0 μ. The average size is 3.0 by 2.5 μ, and the minimum 2.0 by 2.0 μ.

Numbers of Blood Elements in Normal Raccoon

The numbers of the various types of cells in raccoon blood are recorded in Tables I–X. The raccoon are grouped according to age, ranging from under three months to adults one year and over. The same technique and system were used in expressing the red blood corpuscles, haemoglobin content, color index, total white blood corpuscles, lymphocyte, monocyte, neutrophile, eosinophile and basophile leucocytes, the minimum, maximum and mean figures, the standard of deviation and coefficient of variation for raccoon blood, as were used in a previous paper (2).

TABLE I

NUMBERS OF THE BLOOD ELEMENTS IN RACCOON UNDER THREE MONTHS OF AGE

Males				Females			
No.	R.b.cs.	Hb. gm.	Ind.	No.	R.b.cs.	Hb. gm.	Ind.
1	7.752	5.5	0.23	1	8.280	5.0	0.21
2	8.008	5.5	0.23	2	8.280	4.9	0.20
3	9.296	6.7	0.25	3	6.296	4.8	0.26
4	9.528	6.0	0.20	4	7.136	5.2	0.25
5	8.672	6.3	0.23	5	7.968	5.3	0.22
6	8.896	6.6	0.24	6	8.288	5.5	0.23
7	7.944	6.0	0.25	7	7.856	5.8	0.24
8	6.976	5.1	0.24	8	8.376	5.9	0.25
—	—	—	—	9	7.928	6.0	0.25
—	—	—	—	10	8.424	6.5	0.27
—	—	—	—	11	8.352	5.6	0.23
—	—	—	—	12	8.752	5.6	0.21
Min.	6.976	5.1	0.20	Min.	6.296	4.8	0.21
Max.	9.528	6.7	0.25	Max.	8.752	6.5	0.27
Mean	8.384	5.96	0.23	Mean	7.995	5.5	0.23
S.D.	±0.806	±0.6	±0.04	S.D.	±0.640	±0.5	±0.06
C. of V.	10	10	17	C. of V.	8	9	26

Red Blood Corpuscles

The average number of red blood corpuscles per cubic millimetre of raccoon blood in all the groups examined was, males 10,324,000; females 9,749,000.

A maximum number of 13,368,000 for males occurred in a raccoon between seven and twelve months of age. The maximum number for the females, 13,592,000, occurred in an adult. The minimum number in the male groups, 6,976,000, was found in a raccoon under three months of age. The maximum number in all the female groups, 6,296,000, was also found in a raccoon under three months old.

TABLE II

NUMBERS OF THE BLOOD ELEMENTS IN RACCOON UNDER THREE MONTHS OF AGE

Males							Females						
No.	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.	No.	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.
1	16400	55.0	0.5	40.0	4.5	0.0	1	12400	56.5	0.5	40.5	2.5	0.0
		9020	82	6560	738	000			7006	62	5022	310	000
2	10400	50.5	1.0	44.0	4.5	0.0	2	19000	—	—	—	—	—
		5252	104	4576	468	000			—	—	—	—	—
3	6800	55.0	0.0	44.0	1.0	0.0	3	10600	72.5	0.0	25.0	2.5	0.0
		3740	000	2992	68	000			7685	000	2650	265	000
4	9000	65.6	2.0	31.5	1.0	0.0	4	14600	51.0	0.0	46.0	3.0	0.0
		5895	180	2835	90	000			7446	000	6716	438	000
5	7400	53.0	2.5	43.5	1.0	0.0	5	14000	66.0	1.0	31.0	1.5	0.5
		3922	185	3219	74	000			9240	140	4340	210	70
6	9600	54.0	1.0	44.0	1.0	0.0	6	11800	48.0	0.0	46.0	6.0	0.0
		5184	96	4224	96	000			5664	000	5428	708	000
7	8600	48.0	0.0	51.0	1.0	0.0	7	14600	53.0	0.0	43.5	3.5	0.0
		4128	000	4386	86	000			7738	000	6351	511	000
8	8000	45.5	0.0	53.0	1.5	0.0	8	17400	53.0	0.5	36.0	10.5	0.0
		3640	000	4240	120	000			9222	87	6264	1827	000
—	—	—	—	—	—	—	9	6600	60.0	0.5	37.0	2.0	0.5
		—	—	—	—	—			3960	33	2475	132	33
—	—	—	—	—	—	—	10	11800	60.0	1.0	38.0	0.5	0.5
		—	—	—	—	—			7080	118	4484	59	59
—	—	—	—	—	—	—	11	13200	67.0	0.0	30.5	2.5	0.0
		—	—	—	—	—			8844	000	4026	330	000
—	—	—	—	—	—	—	12	8200	49.5	0.5	47.0	3.0	0.0
		—	—	—	—	—			4059	41	3854	246	000
Min.	6800	45.5	0.0	31.5	1.0	0.0	Min.	6600	48.0	0.0	25.0	0.5	0.0
Max.	16400	65.5	2.5	53.0	4.5	0.0	Max.	19000	72.5	1.0	47.0	10.5	0.5
Mean	9525	53.5	0.9	44.0	1.9	0.0	Mean	12850	58.0	0.4	38.2	3.4	0.13
S.D.	±2818	±5.6	±0.9	±6.1	±1.5	±0.0	S.D.	±5392	±7.6	±0.6	±6.9	±2.6	±0.49
C. of V.	30	10	100	14	79	000	C. of V.	42	13	100	2	8	377
Min.		3640	000	2835	68	000	Min.		3960	000	2475	59	000
Max.		9020	185	6560	738	000	Max.		9240	140	6716	1827	70
Mean		5098	81	4129	218	000	Mean		7086	44	4692	461	15
S.D.		±1149	±71	±1117	±283	±000	S.D.		±1762	±49	±1340	±462	±23
C. of V.		23	88	27	130	000	C. of V.		25	111	29	100	153

As both the male and female groups increased in age, the average numbers of red blood corpuscles per cu. mm. of blood increased. The greatest differences occurred in the adult groups where the average number for the males was 11,185,000 with limits of 9,648,000 to 13,264,000; the average number for the females was 11,134,000 with limits of 9,552,000 to 13,592,000.

TABLE III

NUMBERS OF THE BLOOD ELEMENTS IN RACCOON OVER THREE AND UNDER SEVEN MONTHS OF AGE

Males				Females			
No.	R.b.cs.	Hb. gm.	Ind.	No.	R.b.cs.	Hb. gm.	Ind.
1	—	7.4	—	1	9.128	8.5	0.31
2	8.240	9.0	0.37	2	8.152	8.7	0.36
3	7.320	7.7	0.36	3	8.776	7.9	0.29
4	9.344	8.7	0.32	4	—	8.5	—
5	10.360	8.1	0.27	5	7.624	9.1	0.38
6	10.872	9.5	0.29	6	7.568	9.2	0.38
7	10.408	9.9	0.33	7	11.504	8.7	0.24
8	13.296	9.5	0.24	8	9.344	7.9	0.29
9	10.400	9.4	0.31	9	11.752	8.6	0.24
10	9.192	6.3	0.23	10	11.704	8.4	0.23
11	9.464	7.7	0.28	11	10.080	7.7	0.25
12	10.400	9.3	0.31	—	—	—	—
13	9.856	7.9	0.26	—	—	—	—
Min.	7.320	6.3	0.23	Min.	7.568	7.7	0.23
Max.	13.296	9.9	0.37	Max.	11.752	9.2	0.38
Mean	9.929	8.4	0.29	Mean	9.563	8.5	0.29
S.D.	±1.410	±1.0	±0.04	S.D.	±1.549	±0.4	±0.18
C. of V.	14	12	14	C. of V.	16	5	62

Grams of Haemoglobin per 100 cc. of Blood

The average number of grams of haemoglobin per 100 cc. of blood for all the groups examined was, males 9.1; females 8.7 with a maximum of 12.0 for males, which occurred in an adult and in a young male between seven and twelve months of age. The maximum amount for females was 11.5 gm. which was found in an adult. The minimum for males was 5.1 gm. and for females 4.9 gm. occurring in raccoon under three months of age. The haemoglobin content followed the trend of the red blood cells, considering the groups as a whole. The amounts of haemoglobin increased with age in the male groups. In the females the group between seven and twelve months of age showed a slightly higher haemoglobin content than did the adult group. The greatest differences in haemoglobin content occurred in the groups between three and seven months of age where the average for males was 8.4 with limits of 6.3 to 9.9 and the average for females was 8.5 with limits of 7.7 to 9.2.

Color Index

The average color index for all groups examined was .30 for both males

and females. The extreme limits for males were .20 to .40; for females .20 to .39. The color index increased with age in the male groups. In the females the group between seven and twelve months of age showed a higher

TABLE IV

NUMBERS OF THE BLOOD ELEMENTS OF RACCOON OVER THREE AND UNDER SEVEN MONTHS OF AGE

Males							Females						
No.	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.	No.	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.
1	17200	63.0	0.5	35.5	1.0	0.0	1	17200	77.5	0.5	20.0	1.5	0.5
		10836	86	6106	172	000			13330	86	3440	258	86
2	13000	86.5	0.0	13.0	0.5	0.0	2	10800	75.0	1.0	24.0	0.0	0.0
		11245	000	1690	65	000			8100	108	2592	000	000
3	14000	76.0	0.0	22.5	1.0	0.5	3	21400	74.0	0.0	22.0	4.0	0.0
		10640	000	3150	140	70			15836	000	4708	856	000
4	19400	69.5	0.0	28.5	2.0	0.0	4	13400	64.5	1.0	33.0	1.5	0.0.
		13483	000	5529	388	000			8643	134	4422	201	000
5	22000	50.0	1.0	46.5	2.5	0.0	5	14000	63.0	0.5	34.0	2.5	0.0
		11000	220	10230	550	000			8820	70	4760	350	000
6	14800	48.0	0.5	48.5	3.0	0.0	6	16000	85.5	0.0	11.5	3.0	0.0
		7104	74	7178	444	000			13680	000	1840	480	000
7	19400	84.0	0.0	15.0	1.0	0.0	7	29400	63.0	0.5	32.5	3.0	1.0
		16296	000	2910	194	000			18522	147	9555	882	294
8	28600	66.5	0.0	31.0	1.5	1.0	8	13200	63.5	0.0	36.5	0.0	0.0
		19019	000	8866	429	286			8382	000	4818	000	000
9	27800	57.0	0.5	42.5	0.0	0.0	9	8000	63.0	2.0	32.5	2.5	0.0
		15846	139	11815	000	000			5040	160	2600	200	000
10	14200	51.0	0.5	48.0	0.5	0.0	10	17400	76.0	1.0	19.5	3.5	0.0
		7242	71	6816	71	000			13224	174	3393	609	000
11	15800	69.0	0.0	30.0	1.0	0.0	11	28000	32.0	1.5	62.0	4.5	0.0
		10902	000	4740	158	000			8960	420	17360	1260	000
12	32000	60.0	0.5	35.0	4.0	0.5	—	—	—	—	—	—	—
		19200	160	11300	1280	160			—	—	—	—	—
13	16800	45.0	0.5	54.0	0.5	0.0	—	—	—	—	—	—	—
		7560	84	9072	84	000			—	—	—	—	—
Min.	13000	45.0	0.0	13.0	0.0	0.0	Min.	8000	32.0	0.0	11.5	0.0	0.0
Max.	32000	86.5	1.0	54.0	4.0	1.0	Max.	29400	85.5	2.0	62.0	4.5	1.0
Mean	19600	63.5	0.3	34.6	1.4	0.15	Mean	17164	67.0	0.73	29.8	2.4	0.14
S.D.	±5948	±12.8	±0.1	±12.4	±1.1	±0.9	S.D.	±6658	±11.8	±0.49	±12.8	±1.4	±0.33
C. of V.	30	20	33	36	79	600	C. of V.	39	18	67	43	58	236
Min.		7104	000	1690	000	000	Min.		5040	000	1840	000	000
Max.		19200	220	11815	1280	286	Max.		18522	420	17360	1260	294
Mean		12336	64	6877	306	40	Mean		11139	118	5408	463	35
S.D.		±3966	±70	±3132	±326	±84	S.D.		±3844	±113	±4218	±382	±86
C. of V.		32	109	46	106	210	C. of V.		34	96	71	82	246

TABLE V

NUMBERS OF THE BLOOD ELEMENTS IN RACCOON OVER SEVEN AND UNDER TWELVE MONTHS OF AGE

Males				Females			
No.	R.b.cs.	Hb. gm.	Ind.	No.	R.b.cs.	Hb. gm.	Ind.
1	13.368	9.7	0.25	1	9.120	9.7	0.36
2	11.368	12.0	0.36	2	10.352	9.5	0.32
3	11.816	9.5	0.29	3	10.424	11.2	0.37
4	12.344	11.9	0.33	4	9.648	10.5	0.39
5	10.400	9.4	0.31	5	10.928	11.0	0.37
—	—	—	—	6	11.344	10.8	0.33
Min.	10.400	9.4	0.25	Min.	9.120	9.5	0.32
Max.	13.368	12.0	0.36	Max.	11.344	11.2	0.39
Mean	11.799	10.5	0.31	Mean	10.303	10.5	0.36
S.D.	±0.990	±1.2	±0.037	S.D.	±0.743	±0.6	±0.06
C. of V.	8	11	12	C. of V.	7	6	17

TABLE VI

NUMBERS OF THE BLOOD ELEMENTS IN RACCOON OVER SEVEN AND UNDER TWELVE MONTHS OF AGE

Males							Females						
No.	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.	No.	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.
1	35000	45.5	3.0	45.5	5.0	1.0	1	36200	48.0	4.0	42.0	5.0	1.0
		15925	1050	15925	1750	350			17376	1448	15204	1810	362
2	24200	56.5	4.0	38.5	1.0	0.0	2	28000	57.0	2.0	38.5	2.5	0.0
		13673	968	9317	242	000			15960	560	10780	700	000
3	31000	37.0	1.0	56.5	5.5	0.0	3	28600	53.5	1.5	42.0	3.0	0.0
		11470	310	17515	1705	000			15301	429	12012	858	000
4	27800	54.0	3.0	39.5	3.5	0.0	4	19800	54.5	1.0	43.5	1.0	0.0
		15012	834	10981	973	000			10791	198	8613	198	000
5	22600	60.0	1.0	36.5	2.5	0.0	5	22400	42.5	1.0	52.0	4.0	0.5
		13560	226	8249	565	000			9520	224	11648	896	112
—	—	—	—	—	—	—	6	24600	46.0	3.5	48.5	2.0	0.0
		—	—	—	—	—			11316	861	11931	492	000
Min.	22600	37.0	1.0	36.5	1.0	0.0	Min.	19800	42.5	1.0	38.5	1.0	0.0
Max.	35000	60.0	4.0	56.5	5.5	1.0	Max.	36200	57.0	4.0	52.0	4.0	1.0
Mean	28120	50.6	2.4	44.1	3.5	0.2	Mean	26600	50.3	2.2	44.4	2.9	0.3
S.D.	±4507	±8.3	±1.2	±7.2	±1.6	±0.4	S.D.	±5260	±5.1	±1.2	±4.5	±1.3	±0.9
C. of V.	16	16	50	16	46	200	C. of V.	20	10	5	10	45	300
Min.		11470	226	8249	242	000	Min.		9520	198	8613	198	000
Max.		15925	1050	15925	1750	350	Max.		17376	1448	15204	1810	362
Mean		13928	677	12397	1047	70	Mean		13377	620	11698	824	79
S.D.		±1510	±342	±3670	±602	±140	S.D.		±2829	±432	±1949	±499	±133
C. of V.		11	51	30	57	200	C. of V.		21	70	17	61	168

color index than did the adults. An index of .35 was shown for the group between seven and twelve months and of .31 for the adult group. The greatest differences occurred in the group between three and seven months of age where the average for the males was .29 with limits of .23 to .37; the average for the females was .29 with limits of .24 to .38.

White Blood Corpuscles

The average number of white blood corpuscles for all groups examined was 17,880 for males, with a maximum number of 35,000 occurring in a raccoon between seven and twelve months of age and a minimum of 6,800 in a raccoon under three months of age. The average number of white blood

TABLE VII

NUMBERS OF THE BLOOD ELEMENTS IN ADULT RACCOON

Males				Females			
No.	R.b.cs.	Hb. gm.	Ind.	No.	R.b.cs.	Hb. gm.	Ind.
1	13.264	11.2	0.29	1	13.496	11.5	0.29
2	12.424	12.0	0.33	2	10.344	10.5	0.35
3	11.160	11.0	0.33	3	11.896	10.8	0.30
4	10.000	11.9	0.40	4	11.552	11.0	0.31
5	9.648	11.2	0.37	5	9.552	9.7	0.36
6	10.616	11.9	0.36	6	9.928	11.0	0.37
—	—	—	—	7	10.592	8.2	0.25
—	—	—	—	8	10.384	10.2	0.34
—	—	—	—	9	10.008	10.0	0.33
—	—	—	—	10	13.592	11.0	0.26
Min.	9.648	11.0	0.29	Min.	9.552	8.2	0.25
Max.	13.264	12.0	0.40	Max.	13.592	11.5	0.37
Mean	11.185	11.5	0.35	Mean	11.134	10.4	0.31
S.D.	±1.281	±0.4	±0.03	S.D.	±1.381	±0.89	±0.13
C. of V.	11	3	8.6	C. of V.	12	9	42

cells found in female raccoon was 18,166, with a maximum number of 36,200 occurring in a raccoon between seven and twelve months of age. The minimum number of 6,600 occurred in a raccoon under three months of age. The greatest differences occurred in the groups between seven and twelve months of age. The highest numbers of white blood cells per cu. mm. of blood occurred in the groups between seven and twelve months of age. In this group the average number for the males was 28,120 with limits of 22,600 to 35,000; the average number for the females was 26,600 with limits of 19,800 to 36,000. The lowest number of white blood cells per cu. mm. of blood was found in the groups under three months of age. In this group the average number for the males was 9,525 with limits of 6,800 to 16,400, and the average number for the females was 12,850 with limits of 6,600 to 19,000.

Lymphocytes

The average number of lymphocytes for the series of male groups was 7,715 (58.6%) cells per cu. mm. of blood and for the female groups 9,419

(56.1%). The maximum number of lymphocytes found in the male groups was 19,200 (60.0%) in a raccoon between three and seven months of age, and the minimum was 3,640 (53.0%) found in a raccoon under three months of age. The maximum number for the female groups was 18,522 (63.0%) found in a raccoon between three and seven months of age, and the minimum number was 3,960 (60.0%) found in a raccoon under three months of age. The highest average number of lymphocytes per cu. mm. of blood for the groups was, males 13,928 (50.6%) found in the group between seven and twelve months of age, with limits of 11,470 (37.0%) to 15,925 (60.0%); females 11,377 (50.3%) which was also found in the group between seven

TABLE VIII

NUMBERS OF THE BLOOD ELEMENTS IN ADULT RACCOON

Males							Females						
No.	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.	No.	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.
1	12200	78.5 9577	0.5 61	19.5 2379	1.5 183	0.0 000	1	26800	46.0 12328	3.5 938	46.5 12462	4.0 1072	0.0 000
2	14800	59.0 8732	2.0 296	37.5 5550	1.5 222	0.0 000	2	16200	54.5 8829	1.0 162	43.0 6966	1.5 243	0.0 000
3	15600	68.5 10686	1.0 156	23.5 3666	7.0 1092	0.0 000	3	15000	40.5 6075	0.5 75	57.5 8625	1.5 225	0.0 000
4	13000	63.0 8190	2.0 260	30.0 3900	5.0 650	0.0 000	4	18600	65.5 12183	0.0 000	30.5 5673	4.0 744	0.0 000
5	14000	72.0 10080	2.0 280	23.0 3220	3.0 420	0.0 000	5	12400	35.0 4340	0.0 000	60.5 7502	4.5 558	0.0 000
6	16200	60.0 9720	0.0 000	33.5 5427	6.5 1053	0.0 000	6	13400	52.0 6968	0.0 000	43.0 5762	5.0 670	0.0 000
—	—	— —	— —	— —	— —	— —	7	13600	— —	— —	— —	— —	— —
—	—	— —	— —	— —	— —	— —	8	14400	53.0 7632	0.0 000	39.0 5616	8.0 1152	0.0 000
—	—	— —	— —	— —	— —	— —	9	20000	43.0 8600	1.0 200	50.0 10000	6.0 1200	0.0 000
—	—	— —	— —	— —	— —	— —	10	10600	54.0 5724	1.0 106	41.0 4346	4.0 424	0.0 000
Min.	12200	59.0	0.0	19.5	1.5	0.0	Min.	10600	35.0	0.0	30.5	1.5	0.0
Max.	16200	78.5	2.0	37.5	7.0	0.0	Max.	26800	65.5	3.5	60.5	8.0	0.0
Mean	14300	66.8	1.25	27.8	4.1	0.0	Mean	16100	49.3	0.8	45.7	4.3	0.0
S.D.	±1398	±6.9	±0.8	±6.3	±2.2	±0.0	S.D.	±4416	±8.1	±1.0	±8.2	±1.9	±0.0
C. of V.	10	10	64	23	54	000	C. of V.	27	16	125	18	44	000
Min.		8190	000	2379	183	000	Min.		4340	000	4346	225	000
Max.		10686	296	5550	1092	000	Max.		12328	938	12462	1200	000
Mean		9498	176	4024	603	000	Mean		8075	165	7439	699	000
S.D.		±1816	±113	±1135	±365	±000	S.D.		±2597	±282	±2409	±354	±000
C. of V.		19	64	28	61	000	C. of V.		32	171	32	51	000

and twelve months of age, with limits of 9,520 (42.5%) to 17,376 (57.0%). In the raccoon groups under one year of age the total number of lymphocytes per cu. mm. of blood tends to increase with the age of the animal, and decreases after the adult age is reached.

Monocytes

The average number of monocytes occurring in the blood of male raccoon of all the groups examined was 249 (1.2%) with extreme limits of 0 to 1,050 (3.0%). The average for females was 237 (1.3%) with limits of 0 to 1,448 (4.0%). The highest average number of monocytes for a group was found in the groups between seven and twelve months of age where the average for the males was 677 (2.4%) with limits of 226 (1.0%) to 1,050 (4.0%); the average for the female group was 620 (2.2%) with limits of 198 (1.0%) to 1,448 (4.0%).

Neutrophiles

The average number of neutrophiles for male raccoon groups examined was 12,397 (44.1%); and for the females 11,698 (44.4%). The extreme limits for the males were 1,690 (13.0%) to 15,925 (45.5%). Limits for females were 1,840 (11.5%) to 15,204 (42.0%). The highest average number found in a group was in the raccoon between seven and twelve months of age, where the average number for the male group was 12,397 (44.1%) with limits of 8,249 (36.5%) to 15,925 (56.5%); the average for the female group was 11,698 (44.4%) with limits of 8,613 (38.5%) to 15,204 (52.0%). The lowest average number of neutrophiles occurring in the male groups was in the adults, where 4,024 (27.8%) with limits of 183 (19.5%) to 1,092 (37.5%) were found. In the female groups, the lowest number was found in the group under three months of age where the average was 7,309 (39.8%) with limits of 4,692 (29.8%) to 11,698 (45.7%).

TABLE IX

FINAL MEANS FOR THE BLOOD ELEMENTS IN ALL GROUPS OF RACCOON

Males				Females			
Age, months	R.b.cs.	Hb. gm.	Ind.	Age, months	R.b.cs.	Hb. gm.	Ind.
0–3	8.384	6.0	0.23	0–3	7.995	5.5	0.23
3–7	9.929	8.4	0.29	3–7	9.563	8.5	0.29
7–12	11.799	10.5	0.32	7–12	10.303	10.5	0.35
Adults	11.185	11.5	0.35	Adults	11.134	10.4	0.31
Min.	8.384	6.0	0.23	Min.	7.995	5.5	0.23
Max.	11.799	11.5	0.35	Max.	11.134	10.5	0.35
Mean	10.324	9.1	0.30	Mean	9.749	8.7	0.30
S.D.	±1.307	±2.1	±0.08	S.D.	±1.155	±2.0	±0.04
C. of V.	13	23	27	C. of V.	12	23	13

Eosinophiles

The average number of eosinophiles occurring in the blood of male raccoon of all the groups examined was 544 (2.7%) with limits of 218 (1.4%) to 1,047 (4.1%). The average for females was 611 (3.3%) with limits of 458 (2.4%) to 824 (4.3%). The highest average number for a group was found in the group between seven and twelve months of age where the average for the male group was 1,047 (3.5%) with limits of 242 (1.0%) to 1,750 (5.5%). The average number for the female group was 824 (2.9%) with limits of 198 (1.0%) to 1,810 (4.0%). The lowest average number for a group was found in the raccoon under three months of age, where the average for the male group was 218 (1.9%) with limits of 68 (1.0%) to 738 (4.5%); the average number for the female group was 461 (3.4%) with limits of 59 (0.5%) to 1,827 (10.5%).

Basophiles

The average number of basophiles per cu. mm. of blood for all groups examined was, males 28 (0.1%) with limits of 0 to 70 (0.2%); females 32 (0.1%) with limits of 0 to 79 (0.3%). The highest average number of basophiles for a group was found in raccoon between seven and twelve months of age. In this group the average number for the males was 70 (0.2%) with limits of 0 to 350 (1.0%); the highest average number for the females was

TABLE X

FINAL MEANS FOR THE BLOOD ELEMENTS IN ALL GROUPS OF RACCOON

Males							Females						
Age, months	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.	Age, months	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.
0–3	9500	53.5	0.9	44.0	1.0	0.0	0–3	12900	58.0	0.4	38.2	3.4	0.13
		5098	81	4129	218	000			7086	44	4692	458	15
3–7	19600	63.5	0.3	34.6	1.4	0.2	3–7	17164	67.0	0.7	29.8	2.4	0.14
		12336	64	6877	306	40			11139	118	5408	463	35
7–12	28120	50.6	2.4	44.1	3.5	0.2	7–12	26600	50.3	2.2	44.4	2.9	0.3
		13928	677	12397	1047	70			11377	620	11698	824	79
Adults	14300	66.8	1.3	27.8	4.1	0.0	Adults	16000	49.3	0.8	45.7	4.3	0.0
		9498	176	4024	603	000			8075	165	7439	699	000
Min.	9500	50.6	0.3	27.8	1.4	0.0	Min.	12900	49.3	0.4	29.8	2.4	0.0
Max.	28120	66.8	2.4	44.1	4.1	0.2	Max.	26600	67.0	2.2	45.7	4.3	0.3
Mean	17880	58.6	1.2	37.6	2.7	0.1	Mean	18166	56.1	1.3	39.8	3.3	0.14
S.D.	±6908	±7.1	±1.5	±6.9	±1.1	±0.1	S.D.	±5112	±14.2	±0.7	±6.3	±0.7	±0.1
C. of V.	39	12	125	18	41	100	C. of V.	28	25	54	16	21	71
Min.		5098	64	4024	218	000	Min.		7086	44	4692	458	000
Max.		13928	677	12397	1047	70	Max.		11377	620	11698	824	79
Mean		7715	249	6857	544	28	Mean		9419	237	7309	611	32
S.D.		±4183	±250	±3360	±324	± 30	S.D.		±1874	±225	±2727	±157	±30
C. of V.		54	100	48	60	107	C. of V.		20	95	37	26	94

79 (0.3%) with limits of 0 to 362 (1.0%). Basophiles were not observed in the blood of any of the raccoon in either the male or female adult groups, or in the male group under three months of age. In all the groups examined there were a number of raccoon in whose blood basophiles were not observed.

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OSCILLATIONS IN THE MAGNETO-IGNITION SYSTEM AND THEIR ELIMINATION[1]

By Wilbert B. Smith[2]

Abstract

A theoretical analysis of the magneto-ignition system operated in air shows that there are 10 possible oscillations, but an oscillographic analysis shows that in the range tested there are only two actually present, one of frequency about 3500 cycles per second and the other about 180×10^3 cycles per second; the latter is responsible for most of the radio interference. Series resistances, "suppressors", were found to reduce the interference and, when combined with good shielding, very nearly eliminated it, but when large values of series resistance were used the brilliancy of the spark was greatly reduced. This was overcome by increasing the electrostatic capacity of the spark plug by a special design, so that, when the plug was used with a 50,000 ohm suppressor and adequate shielding, there was negligible interference with only a slight reduction in the brilliancy of the spark in air.

Introduction

The high-tension magneto-ignition system is essentially a device for changing mechanical energy into heat energy and releasing it at a certain predetermined time. Although the principle of operation of the system is relatively simple, a complete analysis is extremely complicated owing to the many uncertain factors which enter into its performance.

The successful operation of any ignition system involves sudden changes of currents and voltages, hence a great variety of transient oscillations is possible. It is the purpose of this paper to give special attention to these oscillations and in particular to those responsible for radio interference.

General Description

The constants of the magneto used in these investigations are given in Table I. It was driven normally at 900 r.p.m. through a gear train from the synchronous motor which operated the timing contacts for a General

TABLE I

Circuit constants

Quantity	Value	Unit	Remarks	Quantity	Value	Unit	Remarks
N_1	273	Turn	—	C_3	100	$\mu\mu f.$	Value at 3500 cycles per sec.
L_1	0.02	Henry	Practically constant				
R_1	0.11	Ohm	D-c. resistance		65	$\mu\mu f.$	Value at 25×10^6 cycles per sec.
C_1	0.041	$\mu f.$	Mica condenser				
K	0.97		Computed from resonance	C	5	$\mu\mu f.$	AC spark plug
M	0.75	Henry	Computed from resonance		10	$\mu\mu f.$	Oleo spark plug
N_2	13700	Turn	—	L	10^{-6}	Henry	Computed approximately
L_2	30	Henry	Practically constant	a	3600	Volt	AC spark plug
R_2	3000	Ohm	D-c. resistance	b	5×10^8	Ohm	Estimated approximately with oscillograph
	7×10^6	Ohm	Effective resistance at 3500 cycles per sec.				

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Electric cathode-ray oscillograph. Leads from the magneto to the spark plug were of ordinary ignition cable except where otherwise noted. The spark plugs used were: an AC automobile plug, an Oleo aviation plug, and a special plug to be described later.

Low-frequency oscillograms were taken on a Westinghouse six element oscillograph, and high-frequency traces were taken on a G.E. cathode-ray oscillograph—directly when possible or by the judicious use of vacuum tube amplifiers when either the voltages or currents were beyond the range of the instruments.

Other apparatus included a Burt cathode-ray oscilloscope with which a visual study of the wave forms was made, a three tube radio set used for monitoring the interference, and miscellaneous apparatus used in determining the magneto constants.

The electrical circuit is shown diagrammatically in Fig. 1. This circuit represents conditions prevailing to a fair degree of accuracy providing that due allowance be made for the variation of circuit constants with current or frequency. Most of these quantities remained practically constant over the range of operation, with the exception of the distributed capacity C_2, the secondary effective resistance R_2, and the spark resistance r. The distributed capacity C_2 was measured by a substitution method and was found to decrease slightly at higher frequencies, having the value 100 $\mu\mu$f. at 3500 cycles per second and 65 $\mu\mu$f. at 25×10^5 cycles per second. The secondary effective resistance was also measured by a substitution method and was found to increase enormously with frequency. These results are plotted in Fig. 2.

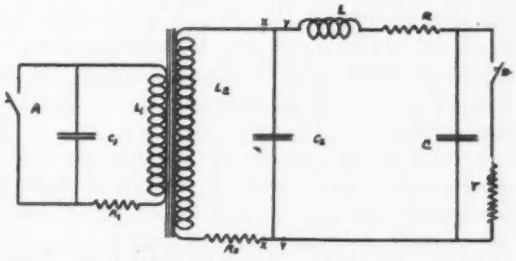

FIG. 1. *Equivalent electrical circuit of the high-tension magneto-ignition system.*

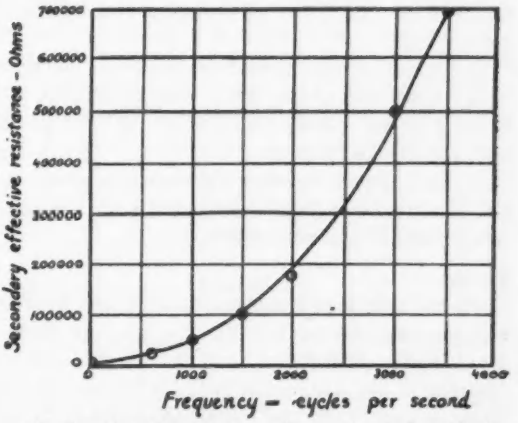

FIG. 2. *Curve showing the variation of secondary effective resistance, R_2, with frequency.*

It was practically impossible to measure the effective spark resistance r owing to its erratic behavior. Therefore, the semi-

empirical formula for the voltage drop across a spark discharge (3),

$$e = a + bi$$

was used, where e is the voltage drop, i the current, and a and b are constants, not necessarily the same values throughout the discharge. Numerical values were used which seemed to be in agreement with experiment.

Table I gives a list of the circuit constants determined for the armature position immediately following the opening of the timing contacts.

Operation

The production of each spark involves a cycle of operations, which for the convenience of analysis and reference may be divided into six rather distinct periods (2), as follows:

1. During the first period the low-voltage winding or primary is short-circuited by the timing contacts, and as the armature rotates, a certain current, I, is generated in the coil. This current stores in the magnetic circuit $\frac{1}{2} LI^2$ joules of energy which later is used to supply current to the spark plug, together with the incidental losses.

2. A few microseconds before a spark is desired at the plug, a cam opens the timing contacts, thus breaking the primary circuit. The primary current decreases rapidly, inducing high voltages in both primary and secondary windings. The high secondary voltage charges the stray secondary capacities to a sufficiently high potential to break down the resistance of the gas between the spark plug electrodes.

3. This period consists of the discharge of the stray secondary capacities through the leads, which are inductive, and the gap. Since the values of inductance and capacity are usually small, and unless suppressors are used, the resistance is also small, oscillations of a very high frequency may take place.

4. Following the discharge of Period 3, the remaining energy in the magnetic and electric circuits is dissipated in the spark between the electrodes of the spark plug.

5. Owing to high speeds or improper adjustments of the timing contacts, it is possible that the latter may close before all the energy has been dissipated in the spark, in which case the energy may divide between the spark and the primary circuit.

6. The circuit remains substantially dead for a considerable time, until the armature has rotated sufficiently for the cycle to begin over again for the production of the next spark.

Period 1

As the magneto armature rotates, the magnetic flux linking the windings changes and induces rotational e.m.f.'s in them. Since the purpose of these e.m.f.'s is to establish a current I in the short-circuited primary prior to the opening of the timing contacts, it follows that, in a well designed magneto, there is practically no rotational voltage for the greater part of the cycle, but just prior to the opening of the timing contacts, the rotational e.m.f.

rises rather suddenly to quite a large value. The oscillogram in Fig. 4 shows the rotational voltage for the magneto used in these tests. The peak value on this wave corresponds to a primary voltage of about 13.5 volts. The lower trace is that of a 60-cycle timing wave.

Period 2

When a spark is desired at the plug, the timing contacts are separated by a cam, thus breaking the primary circuit. Referring to Fig. 1 (omitting all to the right of YY), it is seen immediately that the circuit becomes that of the simple induction coil. From the theory of the induction coil, it follows that there may be two possible frequencies of oscillation if the decrement of the circuit is low, or only one oscillation and one hyperbolic if the decrement is higher, or only hyperbolics if the decrement is very high. Taking the most favorable case, that of low decrement, there will be two frequencies of oscillation which we may call f_1 and f_2, whose numerical values will depend on the circuit constants, chiefly L_1, C_1, L_2, and C_2. From an inspection of the numerical values of these quantities, it is evident that f_1 and f_2 will be of audio frequency, that is, less than 16×10^3 cycles per second.

In the magneto under test, it was found that only one oscillation and one hyperbolic, both with high decrement, occurred. The open-circuit secondary voltage wave is shown in Fig. 9, as taken with the vibrator oscillograph, and in Fig. 8 as taken with the cathode-ray oscillograph. The initial peak value of these waves corresponds to about 9,000 volts. As may be seen (Fig. 8), there is initially an oscillation of large amplitude, frequency 3500 cycles per second and decrement 0.83×10^4, which is followed by a unidirectional component which decreases much more slowly. There were no radio-frequency oscillations of observable magnitude on open circuit.

Period 3

When the voltage across the sparking tips has built up to a sufficient value, the gap breaks down and the stray secondary capacities discharge through the gap. Referring to Fig. 1, breakdown of the gap is the same as closing the switch B. Consider first the circuit consisting of r, C, R, and L (assuming that C_2 acts as a virtual short circuit for high-frequency oscillations). By equating voltages around the circuit, the operational solution of this circuit becomes (1)

$$i = \frac{(E-a)(1+CRP+LCP^2)}{(R+b)+(bRC+L)P+bLCP^2}\,1\,.$$

A possible general solution of this equation is of the form

$$i = Ae^{-\alpha t}\sin(\beta t-\phi),$$

where β has the value given by

$$\beta = \sqrt{\frac{(bRC-L)^2}{(2bLC)^2}-\frac{(R+b)}{bLC}}\,.$$

This is a damped oscillation of frequency $f_3 = \beta/2\pi$. If we substitute typical numerical values in the above equation we find that f_3 is of the order of 7×10^7 cycles per second, which corresponds to a wave-length of about four metres.

Consider next the circuit consisting of r, R, L, and C_2 (assuming that C is negligibly small). The operational solution in this case is,—

$$i = \frac{(E-a)C_2P}{1+(bC_2+RC_2)P+LC_2P^2}\,1,$$

and a general solution is,

$$i = Ae^{-\alpha t}\sin(\beta t-\phi),$$

where β has the value given by

$$\beta = \sqrt{\frac{(b+R)^2}{(2L)^2}-\frac{1}{LC_2}}.$$

This also is a damped oscillation of frequency $f_4 = \beta/2\pi$, which, when suitable numerical values are substituted, is of the order of 2×10^7 cycles per second.

Because of the ultra high frequency of f_3 and f_4, it was impossible to obtain oscillograms of them. However, by means of the radio set, it was possible to confirm the presence of one or both, but it was not possible to determine their exact frequency or magnitude, as both of these characteristics varied considerably. Suffice it to say that the frequencies were of the order indicated above, and that the magnitudes were small compared with that of the f_{10} oscillation of the spark.

When the gap breaks down, there are in addition to the above, transients due to a voltage E applied at the switch B to the circuit consisting of r in series with C_2 and Z in parallel, where Z is the impedance operator for all to the left of XX, Fig. 1. The operational solution in this case works out to be,

$$i = \frac{(C_2PZ+1)E}{C_2bZP+b+Z}\,1.$$

A general solution of the above equation may contain two damped oscillations of frequencies f_5 and f_6. From an inspection of the circuit constants involved, it follows that these two frequencies, when present, will be of the same order of magnitude as f_1 and f_2, namely, audio frequency. In the actual ignition system used in these tests, it was found that no oscillations of this nature took place, those transients which were present being entirely hyperbolic.

Period 4

After the breakdown of the spark gap, with its associated impulse transients, there follows a "steady state", which persists until the energy available is dissipated in the gap. This steady state is characterized by either a steady current flow or a continuous oscillation which is caused by the inherent instability of a spark discharge. The latter oscillation may be called f_{10},

and is shown in Fig. 5, which is a cathode-ray oscillogram of the current flowing through the spark plug, taken by amplifying the voltage drop across a 60 ohm series resistance.

The general shape of the current wave is shown in Fig. 7, which is a low-frequency trace of the current flowing through the AC plug. An extensive visual study of this wave was made with the cathode-ray oscilloscope, and the following points were noted: The current rose suddenly to about 50 ma. when an oscillation of frequency greater than 50 kc. took place (the resolving power of the instrument was about 50 kc.). Following this oscillation the current slowly increased to about 65 ma. maximum, after which it decreased rather rapidly to zero. There was no observable discontinuity or oscillation when the current ceased.

During this period the voltage drop across the spark remained substantially constant. Fig. 10 is a low-frequency trace of the voltage across the plug during sparking. This trace was obtained by operating a UX 245 vacuum tube from a voltage divider across the plug and sending the plate current of the tube directly through the oscillograph element. The excitation of the tube was sufficiently low for its operation to be linear over the operating range. The cathode-ray oscillograph, however, showed this voltage wave to carry a "modulation" corresponding to f_{10}, which caused a variation of 100% in its magnitude.

Referring again to Fig. 5, the oscillation f_{10} appears to be without decrement and is certainly far from sinusoidal. The current appears to jump suddenly to a maximum value and then die out exponentially, the whole cycle being repeated about 180,000 times per second. Altering the external circuit constants seemed to have little effect on either the frequency or amplitude of this oscillation. The only quantities which did seem to influence it were the current flowing and the dimensions of the gap. A reduction of current produced a reduction of oscillation and a decrease of frequency, while a change in gap length produced only a change in frequency. Sphere-gap electrodes seldom gave oscillations of this nature unless the current was abnormally high. Fig. 11 is a cathode-ray oscillogram of the currents flowing in the Oleo plug and in the AC plug. Both of these traces are to the same scale and with the plugs working under identical conditions. The top trace, which is of the Oleo plug, does not show any oscillation of the nature of f_{10}, although such oscillations were occasionally observed visually.

Period 5

It is possible that the timing contacts may close while there is still an appreciable voltage across them, in which case this sudden change of circuit constants may result in further transients. If the total voltage across the timing contacts at the instant of closing be E, the effect is the same as applying $-E$ to the switch A and omitting the condenser C_1 from the circuit in Fig. 1. Considering first the case where there is no spark in progress at the instant

of closing,—that is, all to the right of YY is omitted—the operational solution for e_s, the secondary voltage, may be shown to be

$$e_s = \frac{(R_2 + L_2P)(MC_2P^2)E}{(R_1 + L_1P)(1 + R_2C_2P + L_2C_2P^2) - M^2C_2P^2} 1 .$$

One form of the general solution for this operational solution will be,

$$e_s = Ae^{-\alpha t} + A_1e^{-\alpha_2 t} \sin (\beta_2 t - \phi),$$

which indicates a single possible oscillation of frequency $f_2 = \beta_1/2\pi$. An inspection of the circuit constants shows that, should this oscillation occur, it will most probably be of audio frequency. Actually, in the magneto under test, it was not possible by any normal adjustment or speed variation to cause the contacts to close while there was sufficient voltage across them to produce an observable transient.

If there is a spark in progress when the timing contacts close, then we have the quantity $a+bi$ across the secondary of the magneto. Now, since $a+bi$ is found to be a constant for low frequencies, b must be zero (practically) and hence, if any oscillations were to take place, they would immediately be damped out.

Period 6

If there is a sudden change of voltage or current at the extinction of the spark, a further set of transients may be set up. If the current flowing at the extinction of the spark be I, the mathematical analysis becomes identical with that of the simple induction coil with, of course, the interchange of primary and secondary constants. A general solution of the resulting differential equation may contain two oscillations, which we may call f_7 and f_8, or corresponding hyperbolic terms. These frequencies will of necessity be of the same order of magnitude as f_1 and f_2.

It was found experimentally that, at the extinction of the spark, the current more often died away gradually rather than suddenly as was assumed above. In this case, since the current at extinction is zero, it follows at once that the oscillations f_7 and f_8 will be entirely absent. Referring to Fig. 10, the voltage at the extinction of the spark is seen to increase slightly as the current drops to very low values, after which it follows closely the corresponding portion of the open-circuit curve.

The oscillations appearing in Figs. 9 and 10 are due more to the underdamping of the oscillograph vibrator than to oscillations in the electrical circuit, for no oscillations approaching these amplitudes were observed with the cathode-ray instruments.

The remainder of Period 6 is characterized by the small rotational voltages prior to the repetition of the cycle.

Radio Interference

There are three ways in which a magneto-ignition system may cause interference. First, oscillations and wave forms of audio frequency may affect the radio set by induction or conduction, Second, oscillations of radio frequency may radiate sufficient energy to affect the radio-frequency circuits of the set. Third, extremely sharp wave forms may radiate pulses which may also affect the radio-frequency circuits. Of these, the last two are most important since the radio set is located usually so far from the ignition system that induction is negligible, and paths of conduction are usually absent.

The ideal remedy for any interference is, of course, to eliminate the source. In the case of oscillations due to stray inductances and capacities this is quite feasible, but oscillation of the spark and sharp wave forms offer considerably more difficulty in that there are no equations for these phenomena from which the limiting conditions may be computed.

In the section entitled "Period 3" were developed equations for the two possible impulse oscillations, f_3 and f_4. From an inspection of these equations it is readily seen that for oscillation to take place, the β terms must be imaginary. Hence for non-oscillation these terms must be real; from this we may obtain the limiting conditions.

For f_3 to be absent,

$$R \geqslant 2\sqrt{\frac{L}{C}} + \frac{L}{bC}$$

and for f_4 to be absent,

$$R \geqslant 2\sqrt{\frac{L}{C_3}} - b \ .$$

The above relations justify the use of series resistances commercially known as suppressors, as a means of reducing radio interference. From the above equations, suppressor resistances need only be a few hundred ohms, but it is found in the present case that for satisfactory operation a resistance of about 50,000 ohms is required.

As regards sharp wave forms, very little can be done from a theoretical standpoint. Sharp current waves could be smoothed out by inserting inductance into the circuit, that is, by increasing L. Sharp voltage waves could best be dealt with by careful shielding of all apparatus subjected to these surges.

Since oscillation of the spark, f_{10}, is apparently due only to conditions in the spark, it is evident that we can eliminate this oscillation only by altering these conditions. Unfortunately the only conditions over which there is direct control are the current flowing and the dimensions of the gap, the others being either dependent on these or fixed by conditions of operation.

For the purpose of monitoring the radio interference, use was made of a three tube, 30 to 500 metre radio set, located approximately six feet away from the ignition system. When the ignition system was functioning normally

the interference was so bad that local broadcasting stations were completely drowned out. The intensity of the interference was almost constant over the complete range of the set, being only slightly greater on the shorter waves. There was no noticeable interference when the magneto was operated on open circuit.

A variable resistance was connected in series with the spark plug and its value varied from zero to 50,000 ohms. The interference was observed to decrease steadily as the resistance was increased until it nearly vanished at a value of 40,000 ohms. Increasing the resistance still further had no noticeable effect until a value was reached such that a spark no longer occurred. With no resistance in series the spark was brilliantly white, but as resistance was inserted it became blue and less brilliant until at 50,000 ohms it was a feeble blue line.

A study of the wave forms, made with the cathode-ray oscilloscope, led to the conclusion that the suppressor did not reduce interference by virtue of its damping action but by the great reduction in current which its use involved, the peak current being only about 6 ma. When the original current, peak 65 ma., was forced through the spark by means of higher voltages, the interference was as bad as ever.

Fig. 12 shows the action of a 50,000 ohm suppressor on the oscillations occurring with the AC spark plug. The lower trace is of the amplified voltage drop across a 60 ohm series resistance without the suppressor in the circuit, and shows an oscillation of the spark similar to that appearing in Fig. 5. The upper trace is of the same voltage drop but with a 50,000 ohm suppressor in series. As may be seen, the suppressor has eliminated the oscillation appearing on the lower trace, but there is still present a very small oscillation of frequency about 45 kc. A visual study gave precisely the same results for the Oleo plug.

A concentric shielded cable was tried as a lead from the magneto to the spark plug and this effected a considerable reduction in the interference, but, owing to the unshielded portions at the ends, some energy was still radiated. It was found that as little as an inch of unshielded cable was sufficient to cause objectionable interference. However, when this lead was used with a suppressor at the plug, the interference dropped to a negligible quantity, even though a portion of the cable remained unshielded.

Design of the Special Spark Plug

It appears from the preceding experimental investigations that the radio interference arising from the spark plug spark may be reduced to a satisfactory level, if not eliminated completely, by the use of a 50,000 ohm suppressor resistance in series with the plug, combined with complete shielding.

However, there is the objection to the use of a high resistance in series with the spark that the efficiency of the spark may thereby be reduced. It may be shown mathematically and confirmed experimentally, that the cross-section

area of a spark is closely proportional to the current flowing through it, hence a reduction in current causes a proportionate reduction in the size of the spark. With the suppressor in circuit the maximum current is never much greater than the mean current, hence the size of the spark is more or less determined by this mean current. Without the suppressor there is an initial rush of current, which may be much larger than the mean current, owing to the discharge of the secondary stray capacities through the gap. This large initial current will result in one or both of two things, the area of the spark will be initially large or the temperature will be high, either effect making for a greater efficiency of the spark.

If now a condenser of a value equal to the stray secondary capacities be connected across the spark plug between the suppressor and the gap, it is apparent that the initial conditions will be very nearly the same as without the suppressor. That is, the condenser will charge up through the suppressor resistance until the voltage becomes high enough to break down the gap, whereupon it will discharge through the gap with a current at least as great as that due to the discharge of the stray secondary capacities. This initial current will be followed by the usual small current which the suppressor permits to flow.

With the above considerations in mind, the spark plug shown in section in Fig. 3 was constructed. The right-hand portion is the regulation spark plug base, with the exception of the sphere-gap electrodes. The left-hand portion contains the built in condenser, which consists of a stack of alternate large and small aluminium washers separated by mica washers. The core is of mica and the entire assembly is held together by collars threaded on the central rod. It was decided to use spherical electrodes, since it was found that oscillation of the spark seldom occurred with this type of electrode when the current was of the order found in actual operation.

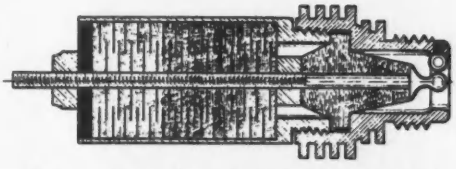

FIG. 3. *Sectional drawing of special spark plug, showing built-in condenser and sphere-gap electrodes.*

Fig. 6 is a cathode-ray oscillogram of the current flowing through this special plug. The upper trace is of the current flowing without the suppressor in the circuit, and the lower one is the same but with a 50,000 ohm suppressor in series. These traces are of the amplified voltage drop across a 60 ohm series resistance. As may be seen, there is at no time a serious oscillation of the spark; the transient which does appear on the upper trace is greatly reduced on the lower one when the suppressor is in circuit. (Compare with Figs. 5, 11, and 12.)

The spark supplied by the special plug was typical of the discharging of a condenser, being brilliantly white and accompanied by the usual snap. There was only a very slight decrease in brilliancy when the suppressor was inserted

in the circuit. The radio interference from this plug was almost as bad as that from the other plugs when no suppressor was used, but with it the interference dropped to a negligible value, and when a shielded cable was used also, vanished entirely.

Acknowledgment

In conclusion the writer wishes to express his gratitude to Dr. H. Vickers, Head of the Department of Electrical Engineering at the University of British Columbia, for his many helpful suggestions and constructive criticisms.

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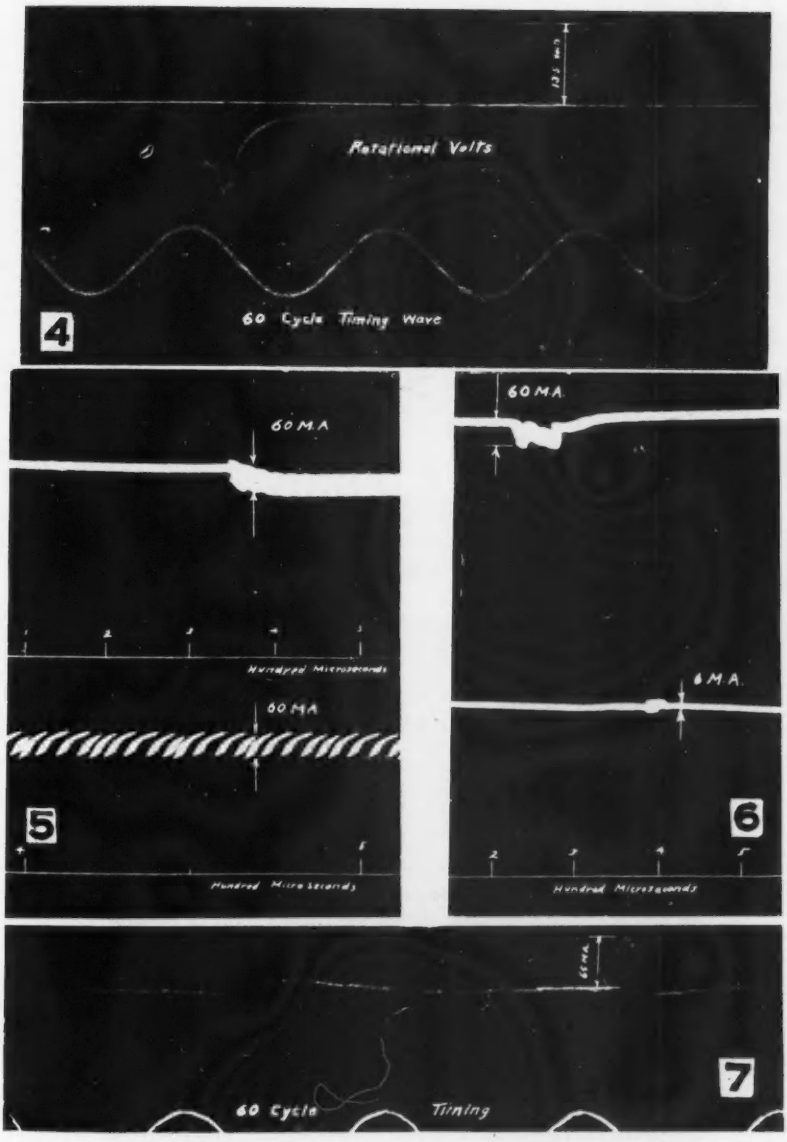


FIG. 4. *Rotational voltage of the magneto.* FIG. 5. *Cathode-ray oscillogram of current flowing through spark plug, showing oscillation of the spark* f_{10}. FIG. 6. *Cathode-ray oscillograms showing currents in special spark plug. Top trace, without suppressor. Bottom trace, with suppressor.* FIG. 7. *Spark plug current wave, taken with vibrator oscillograph.*

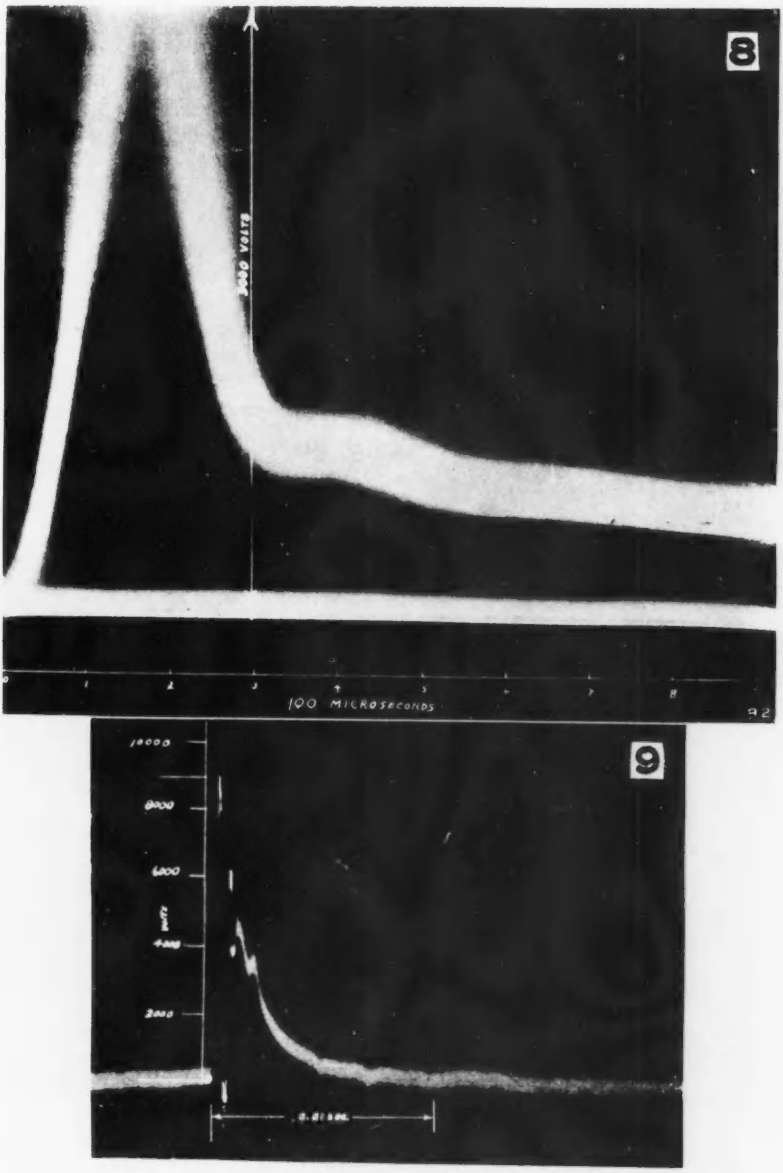
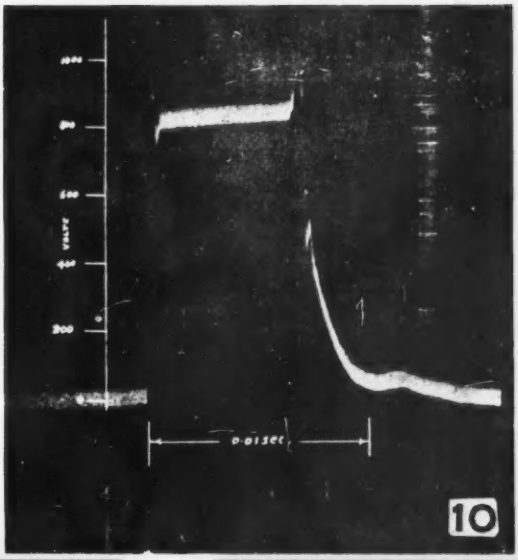
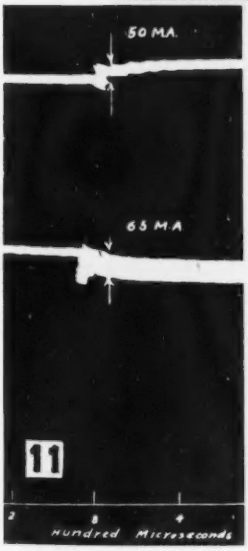


FIG. 8. *Secondary voltage on open circuit, taken with cathode-ray oscillograph.*
FIG. 9. *Secondary voltage on open circuit, taken with vibrator oscillograph.*





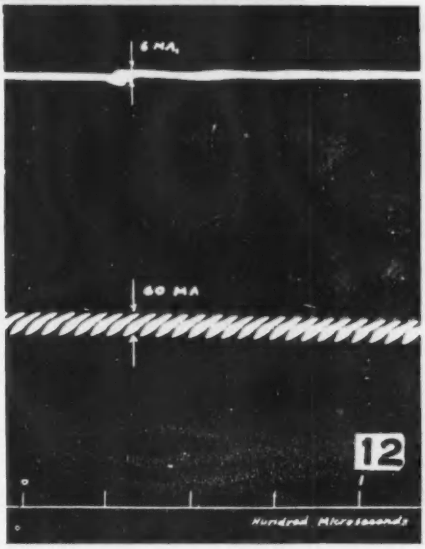


FIG. 10. *Voltage across spark plug during sparking, taken with vibrator oscillograph.* FIG. 11. *Top trace, current flowing in Oleo spark plug. Bottom trace, current flowing in AC spark plug. Both taken with cathode-ray oscillograph.* FIG. 12. *Cathode-ray oscillograms showing the action of a 50,000 ohm suppressor on spark oscillations. Top trace, current in AC plug with suppressor. Bottom trace, current in AC plug without suppressor.*

STUDIES ON THE USE OF FLAME SPECTRA IN CHEMICAL ANALYSIS[1]

By H. W. Lohse[2]

Abstract

Easily constructed accessory equipment for obtaining quantitative flame spectra has been designed, and is discussed. The causes of the variations in the results obtained by the flame spectrum method have been investigated and analyzed. Variations due to manipulation seem to be of little significance except perhaps as regards control of the acetylene pressure. Plate variations are significant and are the chief limiting factor in the accuracy of the method. A comparison of chemical and spectrographic analyses shows that the differences between the results obtained by these two methods are of the same order of magnitude as the differences obtained from plate to plate when the spectrographic method alone is used. Typical analytical data obtained by chemical methods and by the spectrographic method as well are presented.

Introduction

Inasmuch as a rapid and safe method for the estimation of some of the elements of the alkali and alkaline earth groups was required, investigations were made as to the suitability of the spectrographic method for this purpose. This study is based essentially upon the investigations carried out by Lundegårdh (14–18), who successfully developed a quantitative spectrographic method based upon flame spectra obtained by means of an air-acetylene flame.

The properties of flames and the nature of the problem dealt with in general in this paper have been thoroughly discussed elsewhere (2, 3, 4, 10, 24, 25). The general principles and technique of spectrographic methods have been discussed by several authors (1, 5, 8, 9, 12, 19). Specific details of the method of quantitative analysis by means of flame spectra have been given by Lundegårdh (14, 15).

The purpose of the present work was to study the accessory equipment used in quantitative analysis carried out by means of flame spectra, and to test the validity and utility of the data obtained. This paper deals with results obtained from an examination of about 1300 spectrograms, some of which were made for routine work. Such spectrograms are discussed only in so far as they are considered to be of significance as regards the above-mentioned purpose.

Apparatus

The Atomizer

In order to obtain a quietly burning flame, the liquid must be efficiently atomized without having too much air pass through the lamp. Gouy (10) used a glass atomizer, the design of which has had a significant effect upon

[1] *Original manuscript received August 10, 1933.*
Contribution from the MacLennan Laboratory of Physics, University of Toronto, Toronto, Canada, and the Department of Chemistry, Ontario Agricultural College, Guelph, Ontario, Canada. Some of the results presented here are taken from a thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy at the University of Toronto.

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the further development of glass atomizers as undertaken in this study (13), which has involved experimentation with about a score of different types of atomizers. Lundegårdh (15) uses atomizers of hard rubber, silver, gold and platinum. However, no details of design are given by this author. Two of Lundegårdh's silver atomizers have been used in the present investigation.

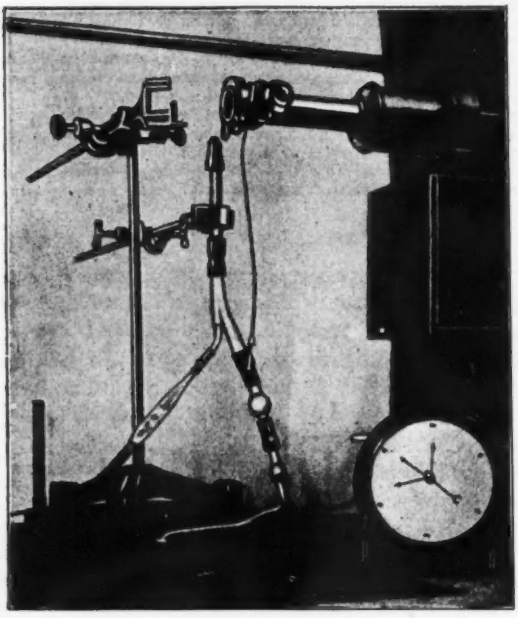

FIG. 1. *Arrangement of vessel and lamp.*

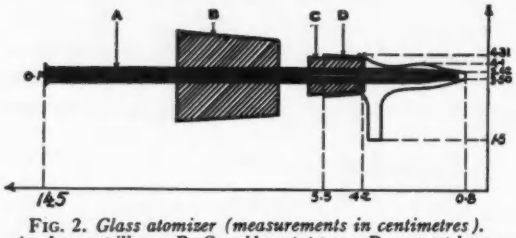

FIG. 2. *Glass atomizer (measurements in centimetres). A, glass capillary; B, C, rubber stoppers; D, cement layer.*

The theoretical aspects of atomization have been discussed by Schweitzer (7), Holroyd (11) and Castleman (6). According to Schweitzer, the atomization of a jet of liquid is most efficient when the jet emerges from the orifice in a turbulent state and when the break-up distance* of the jet is short. Holroyd deduces, on theoretical grounds, that the size of the droplets produced by atomization of a jet of liquid is inversely proportional to the mean angular velocity of the jet, this in turn being inversely proportional to the diameter of the orifice.** On the basis of the investigations referred to above, several types of glass atomizers were studied, and that shown in Fig. 2 was found to fulfil the requirements for atomization under the conditions prevailing in flame spectroscopy.

**The flow of a jet of liquid is said to be turbulent when the paths of the particles of liquid cross one another in a more or less disorderly manner, the particles having various transverse components of velocity. By the "break-up distance" is meant the distance from the nozzle at which the jet breaks up into a cone-shaped spray.*

***Holroyd derives the following equations which express better than words the significant factors which must be taken into consideration in studying atomization for the purpose of developing atomizers. Mean angular velocity* $= \omega = \frac{v}{d}\Psi\left(\frac{\rho v d}{\mu}\right)$, *where* v *= velocity of flow;* d *= diameter of orifice;* μ *= coefficient of viscosity;* ρ *= density of the liquid;* Ψ *= some function of* d, v, μ *and* ρ. *Mean diameter of drops* $= D = \sqrt[3]{k.\lambda/\rho\omega^2}$, *where* λ *= surface tension of liquid and* k *is a constant.*

This atomizer is made entirely of glass. By using a glass capillary as an air inlet, the flow of air is readily regulated in such a way that a fine jet of air of high velocity is produced in the nozzle of the atomizer. This jet acts partly as a jet pump and partly as a means of bringing the liquid into a state of violent turbulence in the nozzle. When the capillary is in the correct position, the jet of liquid should break into spray immediately on leaving the orifice, *i.e.*, the break-up distance should be zero. This position of the capillary is easily found by a little experimentation.* Various degrees of efficiency of atomization are obtained by changing the diameter of the orifice at the end of the tapered capillary and the end of the nozzle of the atomizer tube. The dimensions of the openings of the atomizer shown in Fig. 2 are suitable for lowest to medium efficiency (air pressures from 25 to 90 cm. of mercury.) If very high efficiency of atomization is required, a high air pressure (5–6 atm. or more) is necessary, the capillary being drawn out so that the pointed end is of very fine bore (0.2 mm. or less). By noting the appearance of the flame, the regulation is readily accomplished. The velocity of flow of the air–acetylene–vapor mixture can be varied over a wide range by varying the diameter of the orifice of the quartz tip of the lamp (Fig. 5). The stopper used to hold the capillary in the correct position in the tube is covered with deKhotinsky cement. Care should be taken to see that the capillary is rigidly fixed, and also that the connection between the stopper and the capillary is tightly sealed with the cement, so that no pin holes or crevices (which will retain residues of the liquid atomized) are left. Data obtained in an experiment with two different atomizers and atomizer vessels are shown in Table I.

TABLE I

ATOMIZATION OF DISTILLED WATER**

Atomizer vessel	Atomizer	Gm. of water passing through atomizer in 60 sec.	Mg. of vapor collected in the $CaCl_2$ tube
A (See Fig. 3)	Glass (See Fig. 2)	52.4	141
B (See Fig. 4)	Glass (See Fig. 2)	52.4	129
A	Silver† (from Lundegårdh)	135.2	98
B	Silver (from Lundegårdh)	135.2	96

***Air press., 87 cm. of mercury; temp., 20° C.*

†*This silver atomizer is furnished with an adjustable nozzle and is of medium efficiency (18).*

Lundegårdh reports that, under his earlier experimental conditions, in 60 sec., 15 cc. of water passed through the atomizer used and 37.7 mg. of water vapor passed up into the flame. Klemperer, using an electrolytic atomizer, delivered only 20 mg. to the flame in 60 sec. It should be mentioned that Lundegårdh now uses a platinum atomizer with narrow channels for air and liquid and a high air pressure (6 atm.), whereby very high efficiency of

**The fitting of the capillary in this position is readily accomplished by grinding the point of the capillary on a grindstone.*

atomization is obtained (18). The lower efficiency of the silver atomizer was due, no doubt, to the fact that the flow of liquid was not fully turbulent, and to the fact that this atomizer possessed an appreciable break-up distance.

The Atomizer Vessel

Designs for atomizer vessels are shown in Figs. 3 and 4. The small vessel (Fig. 3) is the most suitable and efficient one when an atomizer with no break-up distance and a short spray is employed, and it should be used when the atomizer shown in Fig. 2 is employed. In addition, it has the further advantage that it is exceedingly convenient to manipulate and is relatively easy to make. When atomizers with an appreciable break-up distance, or a long spray, or both, are used, a larger vessel such as that shown in Fig. 4, or the Lundegårdh vessel, may be employed. This is particularly advisable if a very high air pressure is applied. In Fig. 1 it will be seen that the connection between the vessel and the lamp is made by means of rubber tubing. If this tubing be of a *soft* quality, and of such dimensions that it can be connected to the vessel and lamp without any strain, the connection between these two can be very conveniently made or broken. The larger of the two rubber stoppers (see Fig. 2) used for the support of the atomizer, and for closing the vessel, should also be of soft rubber and of such a size that it fits into the opening in the vessel without any strain. Before use the vessel should be thoroughly cleaned out by means of a cleaning solution of concentrated sulphuric acid and potassium dichromate.

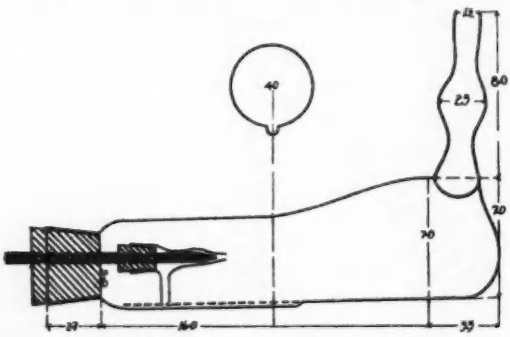

FIG. 3. *Atomizer vessel A (measurements in centimetres).*

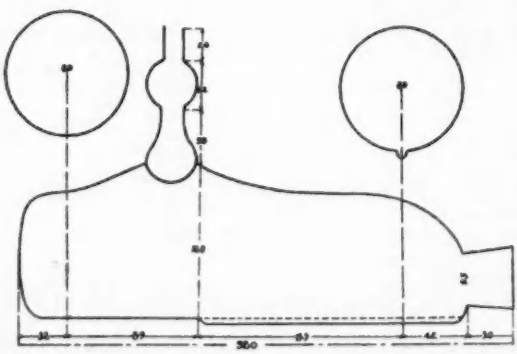

FIG. 4. *Atomizer vessel B (measurements in centimetres).*

The Lamp

The lamp shown in Fig. 5 is so designed that very little condensation of vapor takes place, even during long exposures. It is also very easy to clean. After completion of a series of analyses, the lamp is cleaned by running a

fine jet of water down through the tip, making sure that the sides of the lamp are rinsed all round. The last drops of water adhering to the sides are removed by blowing air through the lamp by means of a piece of rubber tubing held *very lightly* against the tip. In order to facilitate this removal, the individual glass parts of the lamp must occasionally be cleaned with cleaning solution. Since the air enters the lamp through the vessel only, the flame should always be started and extinguished while an appreciable current of air is flowing. A stopcock is placed between the acetylene valve and the manometer. When shutting off the gas, this stopcock is used, the acetylene valve remaining in the position that it occupied during the preceding exposure. In this way, on starting work again, a readjustment of the acetylene pressure to the previous value is very readily obtained. Should the flame accidentally strike back into the lamp, it need cause no concern as experience has shown that it dies down in the narrow orifice of the acetylene inlet. For further security a glass tube filled with absorbent cotton (see Figs. 1 and 5) is placed just in front on the acetylene inlet to the lamp. The bulb between the atomizer vessel and the lamp (Fig. 5, *b*) serves two purposes: *viz.*, (i) it acts as a drop catcher; and (ii) it serves to damp the rhythmic changes in air pressure produced by the atomizer, so that the gas mixture leaves the orifice of the lamp at a relatively constant pressure, thus producing a constant and quietly burning flame. With a properly adjusted set-up, these rhythmic changes should be practically eliminated.

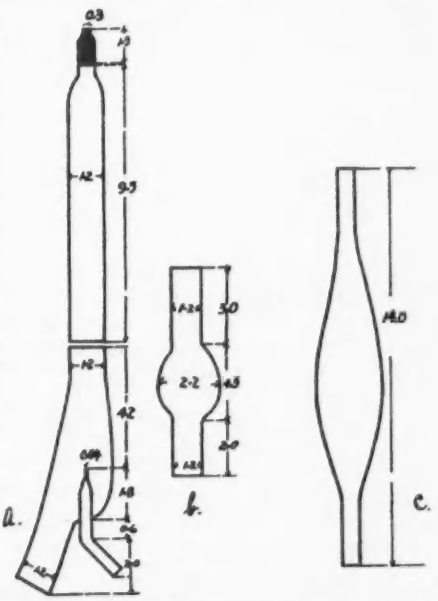

FIG. 5. *Lamp and accessory parts (measurements in centimetres).*

Exposure and Development of the Plates

In this series of experiments, the time of exposure was regulated by means of an iris diaphragm in which the celluloid segments were replaced by thin metal ones, and the mechanism was altered in such a way that exposures of any length of time could be made. With a little experience it is quite possible in this way to stop the exposure in a small fraction of a second. Throughout the experiments, a Hilger spectrograph *E2* with an optical train of quartz was used. With a slit about 0.1 mm. wide it was found that the residual lines due to potassium (4047.2 Å) and manganese (4030.8 Å) were re-

solved.* As these two elements frequently appeared together in the solutions analyzed, this slit width has been used throughout. Owing to the special nature of most of the spectrographic analyses carried out, panchromatic plates (Ilford Soft Gradation Panchromatic, 5 × 25 cm.) have been used. Both metol-hydroquinone and Rodinal (1 : 20, without potassium bromide) were used as developers and both were suitable. The development of these panchromatic plates has been described by Twyman (23). The line intensities were measured on a Moll microphotometer, Type A, adjusted in such a way that deflections over a range of 500 mm. were obtained.**

In developing the designs for the equipment mentioned above it has by no means been the intention of the writer to disregard the accessory equipment designed by Lundegårdh (14) which serves the purpose very well. The spectrographic method possesses such advantages that it can profitably be employed generally, and it is hoped that access to designs for accessory equipment such as can be made in any analytical laboratory will facilitate its employment.

Experimental

The spectrographic method of analysis, in common with all methods of measurement, is burdened with sources of variation† which limit the accuracy of the method. Generally speaking, the variations in the results obtained by the spectrographic method are due partly to variations in *(a)* the air pressure, *(b)* the acetylene pressure and *(c)* the time of exposure, and partly to "plate variation"‡ *viz.*, *(i)* uneven transparency of the plate itself, and *(ii)* uneven development of the plate. An analysis of these variations based on experimental data is shown in Tables II to VIII. It should be added that all solutions used in these experiments were kept in paraffined bottles. In this way the possibility of contamination of the solutions by any substance dissolved from the glass of the containers is obviated.

Experimental Conditions Influencing the Line Intensity

The Effect of Different Air Pressures

It is seen in Table II that the variation of the line intensity for a change of 1 cm. of mercury in the air pressure amounts to about 1% for the sensitive Na-line, and to about 0.3 to 0.4% for the less sensitive K-line. The intensity of the blackening of the band varies much less. By comparing the relative intensities of the corrected and uncorrected lines, a good indication of the significance on the plate variation is obtained (see also the following series). It is possible to control the air pressure by means of a suitable pressure valve to within 0.1 to 0.2 mm. of mercury. In this way, variations due to changes in air pressure are reduced to a negligible amount.

** The large dispersion of this spectrograph should be noted when comparing the results presented here with those obtained by using spectrographs of lower dispersion.*

*** With this arrangement, the reproduction of the readings was very satisfactory.*

† The term variation is used throughout in this paper instead of the term error, because only systematic variations lead to actual errors.

‡ Lundegårdh assumes (15) that the chief cause for the variation in the data obtained by his spectrographic method is due to "plate errors", viz., variation due to heterogeneity of the developed photographic plate itself. This variation is in this paper referred to as "plate variation."

TABLE II

THE EFFECT OF DIFFERENT AIR PRESSURES UPON THE LINE INTENSITY

Air pressure, cm. Hg.	Relative intensity					
	Na-line (5890.2 Å, 5896.2 Å), 0.006 mg. Na per cc.					
	Experiment I			Experiment II		
	Band	Na-line uncorrected	Na-line corrected†	Band	Na-line uncorrected	Na-line corrected
70	100	100	100	100	100	100
80	97	85	91	98	87	92
90	97	79	82	96	77	80
	K-line (4047.2 Å), 0.782 mg. K per cc.					
	Experiment I			Experiment II		
	Band	K-line uncorrected	K-line corrected	Band	K-line uncorrected	K-line corrected
70	100	100	100	100	100	100
80	96	94	98	100	97	97
90	93	87	92	99	93	94

† *The correction for plate variation is carried out according to Lundegårdh (15), using the equation* $I = I_1 . n/n_1$ *(I = corrected line intensity,* I_1 *= uncorrected line intensity,* n_1 *= band reading,* n *= average of all bands.*

NOTE.—*Exposure, 1 min.; acetylene pressure, 34 cm. of water.*

The Effect of Time of Exposure

Considering the fact that the shutter used takes only a fraction of a second to open or close, and thus delimits the exposure very sharply, it is seen from Table III that the effect of the small variations present is negligible. By increasing the time of exposure, considerable increase in the line intensity is obtained, especially for the most sensitive line (calcium).

TABLE III

EFFECT OF TIME OF EXPOSURE ON THE RELATIVE LINE INTENSITY

Time of exposure, min.	Relative line intensities								
	Ca-line (4226.7 Å); 0.0401 mg. Ca per cc.			K-line (4047.2 Å)*; 0.1955 mg. K per cc.			Ba-line (5535.5 Å); 0.328 mg. Ba per cc.**		
	Band	Ca-line uncorrected	Ca-line corrected	Band	K-line uncorrected	K-line corrected	Band	Ba-line uncorrected	Ba-line corrected
1	100	100	100	100	100	100	—	—	—
3	111	90	84	98	90	92	100	100	100
5	113	80	73	93	79	85	93	98	96
10	108	62	59	92	71	77	94	101	93

* *The potassium lines were produced under conditions which differed somewhat from those under which the other two lines were produced.*

** *The plate used was rather old.*

NOTE.—*Air press., 88 cm. of mercury; acetylene press., 34 cm. of water.*

The Effect of Different Acetylene Pressures

The standard deviation of a single flame band is computed by the formula $m = \pm \sqrt{\Sigma d^2/n - 1}$, where n is the number of bands and d the deviation from the mean. The dispersion about the mean is computed by the formula $M = \pm \sqrt{\Sigma d^2/n(n-1)}$, where n and d have the same meanings as above. These numerical dispersions are finally computed in percentages of the mean of all the replications. These two measures of variation are also used in Tables V, VI and VII.

Although the data in Table IV are based upon experiments in which there existed a considerable plate variation, nevertheless there is a sufficiently high correlation between acetylene pressure and line intensity to make it evident that a variation of 1 cm. of water in the acetylene pressure may produce a variation of several per cent in the line intensity of an element whose residual line is very sensitive (calcium, sodium). This is not in agreement

TABLE IV

THE EFFECT OF DIFFERENT ACETYLENE PRESSURES ON THE RELATIVE LINE INTENSITY

Acetylene pressure, cm. water	Relative line intensities					
	Na-line (5890.2 Å; 5896.2 Å); 0.0006 mg. Na per cc.					
	Experiment I			Experiment II		
	Band	Na-line, uncorrected	Na-line, corrected	Band	Na-line, uncorrected	Na-line, corrected
35	100	100	100	100	100	100
34	101	101	100	100	94	99
33	100	97	97	98	100	96
32	99	97	98	99	99	98
	Ca-line (4226.7 Å); 0.0401 mg. Ca per cc.					
	Experiment I			Experiment II		
	Band	Ca-line, uncorrected	Ca-line, corrected	Band	Ca-line, uncorrected	Ca-line, corrected
35	100	100	100	100	100	100
34	107	104	102	91	90	100
33	105	102	103	89	87	102
32	99	95	105	86	83	103
	K-line (4047.2 A); 0.782 mg. K per cc.					
	Experiment I			Experiment II		
	Band	K-line, uncorrected	K-line, corrected	Band	K-line, uncorrected	K-line, corrected
35	100	100	100	100	100	100
34	101	100	99	98	97	99
33	101	99	98	91	86	100
32	99	98	99	94	95	101

NOTE.—*Air press., 9 cm. of mercury; time of exposure, 1 min.; diameter of orifice of quartz tip of lamp, 3 mm.*

TABLE V

VARIABILITY IN THE FLAME BANDS ADJACENT TO THE LINES

—	Standard deviation of a single flame band, m, %		Average deviation about the mean, of all flame bands, M, %	
	Experiment I	Experiment II	Experiment I	Experiment II
Na-line	0.8	1.3	0.4	0.7
Ca-line	3.8	9.7	1.9	4.0
K-line	1.0	2.5	0.5	1.4

with Lundegårdh's conclusion (15) that a deviation of 1 cm. of water in the acetylene pressure is not significant.

Variability in the Blackening of the Flame Bands

The plates referred to in Table VI are arranged according to the average galvanometer deflection due to the bands. This arrangement should give a

TABLE VI

VARIABILITY IN THE BLACKENING OF THE FLAME BANDS

Plate No.	Number of spectrograms, n	m, %	M, %	Mean galvanometer deflection, mm.	Spectrograms
61	7	0.7	0.3	483	Soil extracts and KCl standards.
88	6	4.3	2.2	478	Soil extracts and KCl standards.
58	5	3.4	1.6	444	KCl solutions.
69	5	0.2	0.07	425	KCl solutions
68	9	2.9	1.0	372	Soil extracts and KCl standards
70	5	1.4	0.6	360	Soil extracts and KCl standards
90	9	3.9	1.3	343	Feldspar (solution) and KCl standards
66	9	4.0	1.4	287	Soil extracts, phlogopite (solution) and KCl standards
89	9	3.6	0.4	261	Soil extracts, feldspar (solution) and KCl standards
65	9	2.3	0.8	250	Soil extract and KCl standards.

fair indication of the effect of the degree of development on the plate variation. This series of experiments indicates (Table VI) that a certain amount of fogging of the plates does not influence the accuracy of measurement of the line intensities when the light source used is powerful enough to provide for sufficiently large deflections. This is in agreement with the viewpoints of Ornstein, Moll and Burger (19), whereas Lundegårdh (15) considers fogging a significant cause of plate variation.

Variability of the Line Intensity when Different Numbers of Replications were made

The replications shown in Table VII were made in consecutive order across the plate, and the line readings were corrected as described in the footnote to Table II.

TABLE VII

VARIABILITY IN LINE INTENSITY WITH NUMBER OF REPLICATIONS

No. of replications	Na-lines, (5890.2 Å; 5896.2 Å) 0.0006 mg. Na per cc.		Ca-line, (4226.7 Å) 0.0401 mg. Ca per cc.		K-line, (4047.2 Å) 0.782 mg. K per cc.	
	m, %	*M*, %	*m*, %	*M*, %	*m*, %	*M*, %
2	1.6	1.1	3.0	2.1	0.69	0.49
3	0.55	0.00	2.3	1.1	0.56	0.32
4	1.2	0.62	0.76	0.40	1.4	0.70
5	1.9	0.82	1.8	0.79	1.9	0.83
6	2.4	1.0	1.6	0.66	2.4	1.0
7	3.1	1.2	1.5	0.56	2.8	1.1
8	3.5	1.2	1.3	0.15	3.0	1.1
9	3.0	1.7	—	—	3.0	1.7

NOTE.—*Air press., 88 cm. of mercury; acetylene press., 34 cm. of water; exposure, 1 min.; orifice of lamp, 3 mm.*

The variability (for all the replications) in the flame bands adjacent to the lines was as shown in Table VIII.

TABLE VIII

VARIABILITY IN THE FLAME BANDS ADJACENT TO THE LINES (COMPUTED ON A BASIS OF ALL THE REPLICATIONS)

Na-line		Ca-line		K-line	
m, %	*M*, %	*m*, %	*M*, %	*m*, %	*M*, %
5.3	1.9	4.2	1.5	7.3	2.0

That we are dealing here with systematic variation appears from the fact that the dispersion about the mean increases with the number of replications. The dispersions about the mean of the first successive either two or three spectrograms of the calcium line (4226.7 Å) are very high, owing to the fact that the intensity of the line on the second spectrogram deviated greatly. The galvanometer deflections due to the first three replications of the calcium line were 334, 320 and 332, while those due to the last three replications of the calcium line were 332, 332 and 330. In the latter case, both *m* and *M* are negligible. However it does at times (though not often) happen in general routine work, that a line deviates considerably.

COMPUTATION OF RESULTS FROM EXPERIMENTAL DATA

As the plate variation is systematic and causes actual errors, the results obtained by the spectrographic method discussed in this paper should not be computed on the basis of a theoretical standard curve, but rather on the basis of an empirical one. To summarize, the rules to be followed in order

to obtain the most reliable and accurate results are as follows: (i) the results should be computed on the basis of an *empirical* standard curve*; (ii) standards and unknown solutions should be of approximately the same concentration; (iii) the range of concentration used in a series of analyses on the same plate should never be too wide; (iv) the replications should be eventually made on different plates. Even though the plate variation may be different for different types of plates, the above-mentioned rules are probably capable of quite general application. General rules for minimizing the plate variation are not readily given. It seems that slow development and thorough shaking of the developer and fixer (during the first few minutes at least) result in the most uniform development. With panchromatic plates such as those employed here, care should be taken not to use plates that have been stored too long (23).

(For further discussion of the problem dealt with in this paragraph see References (5) and (15).)

Quantitative Analysis

Comparative chemical and spectrographic analyses were carried out intensively by Lundegårdh (14, 15), and, on the average, satisfactory results were obtained. Such comparative analyses have also been carried out during the course of the present studies, the results of which are shown in Table IX.

TABLE IX

COMPARATIVE CHEMICAL AND SPECTROGRAPHIC ANALYSES

Sample	Spectrographic method	Chemical methods	Nature of chemical method	Difference between the two methods, %
1. Orthoclase	Total, 8.56% K	8.54% K 8.87%	Colorimetric Using K_2PtCl_6	0.23 3.60
2. Muscovite	Total, 7.89% K	8.00% K	Using K_2PtCl_6	1.4
3. Feldspar	Total, 9.34% K	9.30% K	Colorimetric	0.43
4. Soil extract	Extracted 480 mg. K per kg. of soil	Extracted 488 mg. K per kg. of soil	Colorimetric	1.70
5. Ba in paper	65.5 mg. Ba in 77.78 gm. of paper	67.4 mg. Ba in 77.78 gm. of paper	Gravimetric	2.90

It is interesting to note that the differences between the results obtained by these two methods are of the same order of magnitude as the band and plate variation discussed above.

**When the standard curve is drawn an appreciable plate variation is readily recognized if present.*

Chemical Analytical Procedures

A suspension of the finely ground potash-bearing mineral (Nos. 1, 2 and 3) in concentrated sulphuric acid, to which hydrofluoric acid was added, was evaporated to dryness. This was repeated until all the silica was removed. Hydrochloric acid was added and the suspension evaporated to complete dryness. This operation was repeated twice. The residue was then taken up with hot dilute hydrochloric acid and filtered through ashless filter paper. The filter paper with the residue (which was very small in all cases) was ashed at as low a temperature as possible, the ash taken up with hot dilute hydrochloric acid, filtered off, and this filtrate added to the main portion of the filtrate. The final residue was almost negligible; it was not fused with sodium carbonate, in order that alkalies might not be introduced. On the basis of experience obtained in other work, it is felt that such a fusion would not, in this case, have influenced the data significantly. The combined filtrates were evaporated to complete dryness, the residues taken up with water and diluted to a volume suitable for the spectrographic and colorimetric measurements which were carried out directly on this solution. By the colorimetric procedure, the potassium was precipitated directly from these solutions by means of sodium cobaltinitrite, prepared according to Biilmann (13), and the potassium was estimated indirectly by determining the cobalt in the precipitate (13). The good agreement between the spectrographic and colorimetric methods may be ascribed to the fact that in both cases the results were obtained by using a fully standardized technique, and by basing the final computation of the results on an empirical standard curve. The same standard solutions were used in both cases. In the two cases where potassium chloroplatinate has been used, the alkali chlorides were very carefully isolated, although no attempt was made to correct for the possible presence of small amounts of caesium and rubidium. This may explain the somewhat higher result obtained when potassium chloroplatinate was used as a precipitating agent.

The soil (No. 4) was extracted with water. To a suitable aliquot of the extract was added a few drops of dilute sulphuric acid, and the solution was evaporated to dryness. Organic matter was burned away at low heat, the residue taken up with water, filtered off, and the filtrate concentrated to a volume suitable for spectrographic and colorimetric analysis.

In the case of the determination of barium* in paper, 77.78 gm. of paper was ashed and a suspension of the ash in concentrated sulphuric acid and in hydrofluoric acid was evaporated to dryness several times to remove silica. The residue was taken up with hydrochloric acid in order to dissolve alumina and other soluble bases. The insoluble residue (mostly barium sulphate) was filtered off and ignited (weight of ash, 0.1639 gm.). This residue was reduced with cane-sugar charcoal and the last traces of insoluble material were fused with sodium carbonate. The hydrochloric acid solution of the melt

*This study was undertaken at the request of the Ontario Research Foundation, Toronto. The chemical work was done by Mr. O. J. Schierholtz, Research Chemist of the Research Foundation, to whom the author is indebted for permission to publish these results.

was evaporated to dryness twice and the volume finally made up to 50 cc. The barium content of this solution was determined spectrographically and by the chemical gravimetric method. It should be mentioned that the acid blank and the acid filtrate containing the soluble bases did not contain any barium (spectrographic test).

The significance of this comparison of the two methods lies in the fact that these examples all represent cases where only very tedious chemical methods lead to reliable results. Also it shows that the spectrographic method serves the purpose of macroanalysis as well as microanalysis.

Acknowledgments

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MICROSCOPIC FEATURES OF THE PHALEN SEAM, SYDNEY COAL FIELD, N.S.[1]

By W. R. Newman[2]

Abstract

The microscopic and chemical nature of the anthraxylon, attritus and fusain of the Phalen coal were studied. The attritus is composed of humain (humic degradation matter), opaque matter, resins, waxes, cuticles and spores. Pyrite and other mineral matter also were detected. The microstructural features of the coals of the Phalen seam are so distinctive that they can be divided into three groups. Coal of Group 1 is characterized by a high spore, low resin, and low pyrite content, and was formed under open-water conditions which existed in the central part of the swamp. Group 3 coal has a low spore, high resin, and high pyrite content, and originated under conditions which prevailed near the shore line of the original swamp. Group 2 is a transition stage between Groups 1 and 3. The microstructure of the coal indicates that future mining possibilities are most favorable in the vicinity of the Bridgeport anticline.

The sulphur in the Phalen seam is due mainly to pyrite and is irregularly distributed throughout the seam. The pyrite was probably formed from iron- and sulphur-bearing solutions which entered the swamp in the vicinity of Cape Percy. The iron and sulphur were precipitated by the organic products of decay, producing a disseminated pyrite in the coal.

Nine types of megaspores and eleven types of microspores were determined on the basis of their morphological appearance. Correlation by means of these spores was not possible, however, owing to the variability in the spore content and the lack of any persistent distinctive spore.

Introduction

The land portion of the Sydney coal field is only the fringe of an extensive coal basin that extends far out beneath the sea. The future of the field depends upon the undersea development of the collieries. In 1928 the Geological Survey of Canada and the Fuel Division of the Mines Branch undertook a joint survey of the Phalen seam, the largest and most important seam in the field. Thirteen locations in the Phalen seam, some inland and some under the sea, were carefully selected, and complete pillars varying from 5.5 to 8.5 ft. in height and 1 ft. square were extracted. These pillars were examined as closely as possible before removal, and were subdivided into 20-24 sections on the basis of their macroscopic appearance. The pillars were carefully packed and shipped to Ottawa where microscopic and chemical examinations of the sections were made.

The writer, under the supervision of Dr. B. R. MacKay of the Geological Survey of Canada, undertook the microscopic investigation of the pillars. The purpose of this study was to determine the nature and constitution of the coal in the Phalen seam, the varying character of the constituents of the coal throughout the field, and the physiographic conditions under which the

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Published with the permission of the Director, Geological Survey of Canada, Ottawa. The investigation was part of a more extensive study of the coal deposits of the Sydney coal basin, N.S., and of the physical constitution of the coals of Canada. This paper is a synopsis of a thesis presented as part of the requirements for the degree of Doctor of Philosophy at the University of Toronto. The investigation was made for the Geological Survey of Canada which supported it financially and provided materials and laboratory facilities.

[2] *Postgraduate student, University of Toronto, and student assistant, Geological Survey of Canada.*

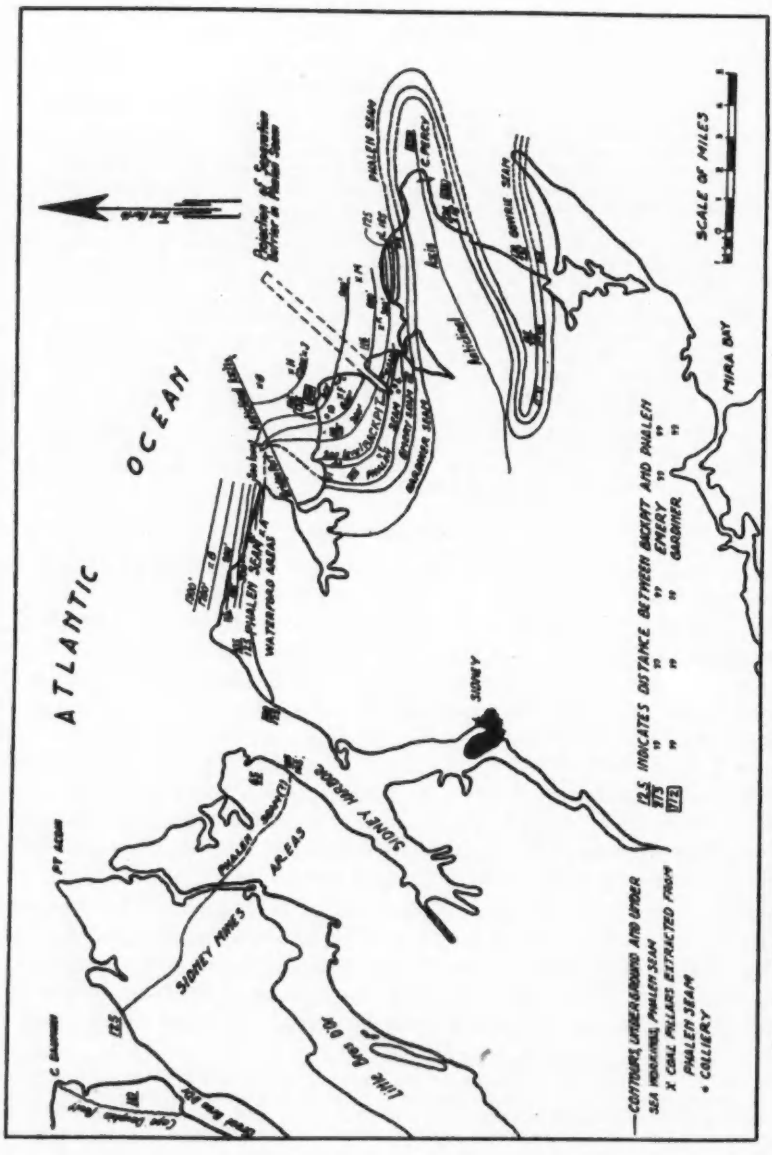


FIG. 1. *Map showing the possible extent of the Phalen seam and its underground and undersea development.*

coal was laid down. In addition, the nature and distribution of the sulphur in the coal, and the possibilities of correlation by means of the spore content of the seam were investigated.

Phalen Coal Seam

The Phalen seam supplies more coal than any other seam in Canada. Stratigraphically, it is part of the *Anthracomya* zone of the Morien series (6) and occurs approximately 150 ft. above the base of the zone. The seam is believed to continue throughout the whole extent of the Sydney field (Fig. 1).

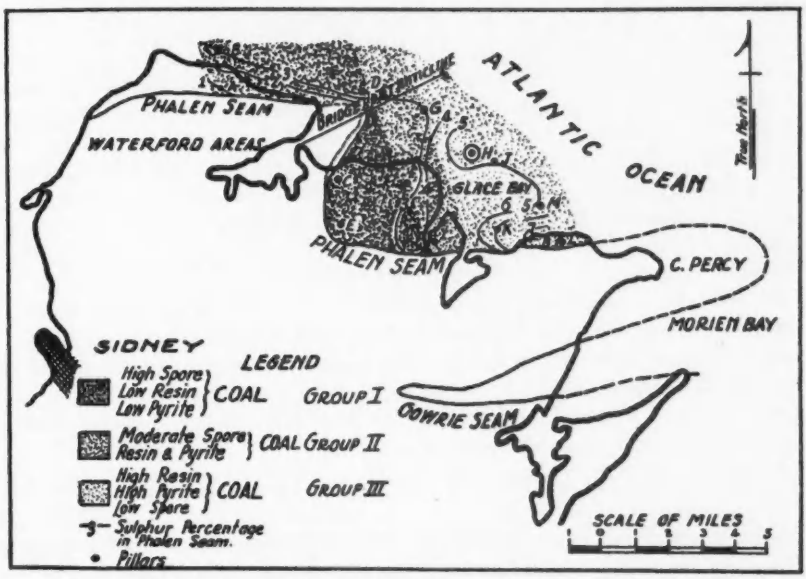


FIG. 2. *Map showing variation in microstructure and sulphur distribution of the Phalen coal seam.*

It follows the structural features of the field, but has been separated by folds into a series of apparently separate seams. The structural features of the Phalen seam are relatively simple. In the Morien basin, it forms the two limbs of a syncline. It has not been traced around the Cape Percy anticline, but it reappears in the Glace Bay region where it dips gently seaward. The Bridgeport anticline is the locale of the best coal in the Phalen seam. In the Lingan basin, the seam dips rather deeply seaward (Fig. 2). The extension of the seam under Sydney Harbor and on the Sydney Mines side has not been proved. The folding of the seam as a whole appears to become less intense seaward.

Macroscopic Description of Phalen Coal

The coal of the Phalen seam is a banded bituminous steam coal, possessing fair coking properties. It has a high sulphur and ash content which varies

in different parts of the seam. In the hand specimen shown in Plate I, Fig. 1, the three normal banded components of bituminous coal are recognized, —anthraxylon or bright coal, attritus or dull coal, and fusain.

The anthraxylon appears as bright black bands with a vitreous lustre. These bands are never more than an inch in thickness and are of a lenticular nature, pinching out laterally. They are uniformly distributed from the top to the bottom of the seam. There is, however, an apparent increase in the proportion of anthraxylon in the eastern portion of the field; it is undoubtedly purer than the other components of the coal, and is relatively free from mineral matter, which occurs mainly in the attritus.

The attritus is the groundmass in which the anthraxylon is embedded, and has a characteristic dull lustre. In the hand specimen it forms the greater bulk of the coal and is varied in structure. In the dull coal, numerous microscopic lenticles of anthraxylon are noticeable. In addition, the dull coal contains the largest proportion of mineral impurities; but mineral matter which is intimately associated with the attritus is not visible macroscopically.

The fusain is a dull, powdery, charcoal-like substance, finely distributed throughout the coal. Considering the fragile nature of the substance, it is strange that delicate fern and leaf impressions in the fusain have been preserved. These structures are especially noticeable in the hand specimen where the fusain is parallel to the bedding. The fusain occurs in thin lenticles about $\frac{1}{8}$ in. or less in thickness. The fusain is more abundant at the top and the bottom of the pillars, and increases greatly in proportion in the eastern part of the field. In most cases, fusain does not appear to contain mineral matter.

The Phalen coal varies from an anthraxylous-attrital to an attrital-anthraxylous type (3, pp. 22-25). The banded structure may be regarded as an alternation of a coarsely and a finely banded type. As the groundmass of the coal is translucent in thin section, the Phalen coal is a normal bituminous or bright coal.

Microscopic Character of the Phalen Seam

Examination of thin sections of coal by transmitted light was the chief method of study used in this investigation. Thiessen's method (3, pp. 22-25) with a few modifications was the one adopted.

The microscopic components of the Phalen coal may be classed under three main headings: anthraxylon, attritus and fusain.

Anthraxylon

Anthraxylon is present throughout the whole seam and occurs in lenticular bodies. Under the microscope, in thin section, it is usually of uniform mahogany color giving little indication of structure (Plate 1, Fig. 2). Cell structure in the anthraxylon was found only in a few cases. Normal, faint cell structure can occasionally be seen. The anthraxylon has been practically

all derived from ordinary woody tissue. It is believed that anthraxylon is composed chiefly of lignin, the cellulose having been largely decomposed. The structureless character of the anthraxylon would indicate that biochemical action had been active in the transformation of cellular plant tissue into the homogeneous anthraxylon. The plant tissue may have been infiltrated by colloidal substances and later transformed into the anthraxylon lenticle.

The coking properties of coal are due mainly to the anthraxylon components. A coal with a high percentage of attrital matter is usually a poorer coking coal than one of an anthraxylous nature. Not all anthraxylous coals will coke, as this property is possessed only by coals in certain stages of coalification.

Attritus

Attritus forms the most interesting and complex part of coal. In its broadest sense, it includes not only all comminuted plant matter contributed from the original swamp, but it may also contain microscopic lenticles of anthraxylon and small particles of fusain. The attritus of the Phalen coal consists of the following constituents: humic degradation matter, spore exines, resins, cuticles and waxes, opaque matter and mineral matter.

Humic Degradation Matter

The term "humic degradation matter" is rather difficult to define, and, as it is an unwieldy expression, the writer here proposes to adopt the term "humain" as synonymous with it. Humain is composed of the fragments of the cell walls of all sorts of plant and woody tissue and the contents of cells of plants (Plate III, Fig. 1). These have been greatly altered by biochemical decay and deposited in the swamp. It generally forms the bulk of the attritus.

Spore Exines

Spores are the reproductive bodies of the lower plants. As yet, not enough is known about spores found in coal to classify them genetically. The classification adopted is strictly morphological and is based on the external appearance of the spores. The American investigators (8) maintain that the limiting sizes for microspores are 13–70 μ, and for megaspores, 500–3000 μ. The English coal microscopists (7, p. 8) find a range of 15–100 μ for microspores, and 130–2500 μ for megaspores. These figures however are subject to change. It must be realized that the size of a spore does not distinguish a microspore from a megaspore as much as do its mode of development and function as a spore in the living plant. The microspores develop in large numbers in a microsporangium (Plate II, Fig. 2), but only one or a few megaspores develop in a megasporangium.

The spores are spherical bodies, but owing to putrefaction the living matter enclosed by the shell, or exine, is lost. The spore on being buried is flattened by pressure. As the exines are extremely resistant to decay, they may become concentrated in the coal when conditions are favorable. In the horizontal sections, the spores are more or less circular, and the tetrad markings may be frequently distinguished. When examined in vertical sections, they appear

as collapsed rings, the living matter within the spore having been decomposed and the spore flattened by pressure. Microspores are by far the more numerous of the two spore types. The megaspores, however, on account of their larger size, are more easily studied (Figs. 3 and 4).

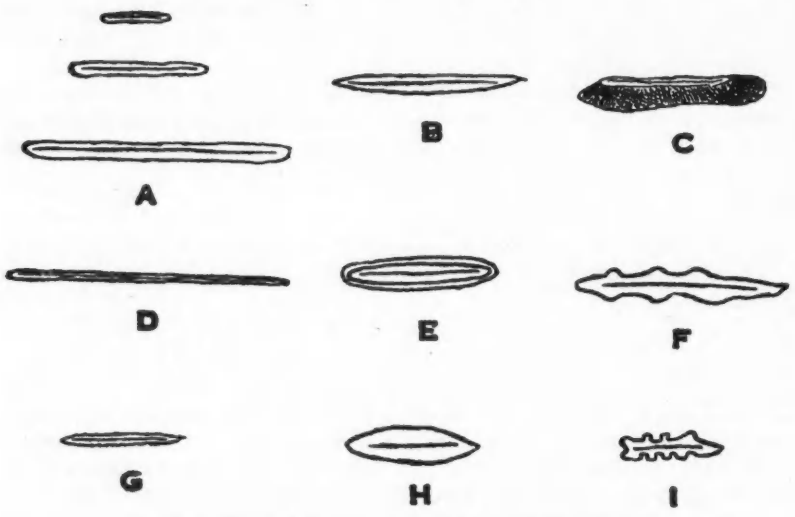


FIG. 3. *Megaspores found in the Phalen coal seam.* × *100.*

The walls of the spores are frequently ornamented and sculptured, and it is on the basis of these ornamentations that the spores are classified. Spores may be serrated or they may be perfectly smooth. They may have wings, spines, tubercles and tufts.

Cannel coals are high-spore coals and are the result of the accumulation of spores which were conveyed by the wind or currents to a swamp, lake, lagoon, or sea where tranquil open-water conditions prevailed. A high-spore coal would thus indicate open-water conditions of deposition. Spores are the important oil- and gas-yielding constituents in coal.

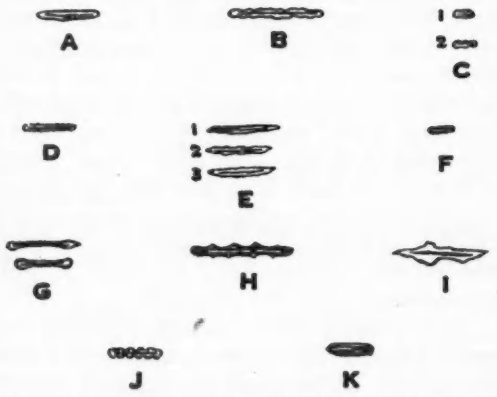


FIG. 4. *Microspores found in the Phalen coal seam.* × *630.*

A description of the microspores and megaspores found in the Phalen seam will be given later.

Resins (11)

Resins are conspicuous bodies in most coals. They are very resistant to putrefaction and to destructive agents of any sort. Resins are the remains of the matter stored up in resin ducts and certain cells of plants. On account of the appearance and mode of occurrence of oval-shaped, reddish-colored bodies in the higher-rank coals, it is assumed by analogy that these are the remains of the original resin in the plants. The abundance and distribution of the resins are an indication of the amount of decay undergone by the original plant material. Resins are usually amongst the last products to survive subaqueous biochemical decomposition. As a result of dehydration, progressive chemical reduction, and the collapse of the plant tissue due to pressure, the resins have been concentrated in coal so that their volume is greatly increased relative to that of the other constituents.

There are three modes of occurrence of resinous bodies in the Phalen seam.

1. *Flattened elongated bands in the anthraxylon.* The resinous bodies were compressed, flattened and elongated in woody tissue which was changed into anthraxylon. This represents the stage of least decay.

2. *Disseminated bodies throughout the attritus.* The resinous bodies begin to increase in proportion, under putrefactive conditions, and are found scattered throughout the attritus with the bodies more or less resistant to decay. This stage is intermediate between the first and third types and is the commonest.

3. *Aggregations of resinous bodies in the attritus.* When putrefaction has reached such a stage that the humic matter has disappeared and only the most resistant bodies remain, aggregations or clusters of resinous bodies are found. This is the stage of greatest decay (Plate II, Fig. 5).

The proportion of resinous bodies varies in different parts of the Phalen seam, thus indicating that conditions of decay were not uniform in different parts of the seam.

Cuticles and Waxes

Closely allied to the resins in nature are the cuticles, As is the case with the present day plants, the Carboniferous flora had certain of their tissues protected with a thin layer of cuticular matter. This waxy material is extremely resistant to putrefaction and, as a result, when plants accumulate in a swamp, it increases in proportion as the more readily decomposed tissues of plants decay. In nearly all coals, fragments of cuticular tissue are common. In thin section, they appear as lemon-yellow elongated bands, characterized by a serrated border (Plate III, Fig. 2). The borders result when the cuticles conform to the cells of the once underlying epidermal tissue.

Oval-shaped bodies of lemon-yellow color, which are believed to be of a waxy nature, are occasionally seen in the Phalen coal (Plate II, Fig. 6). They are similar in shape to the resins, but are not of the same color and do not hold the same relations to the attritus.

Opaque Matter

The nature of the opaque matter is not yet fully understood. It may have been derived from the cell contents of plants. Opaque matter, in places, appears to be a highly altered substance similar to fusain. This is the case in the Phalen seam where the opaque matter appears to be a finely divided variety of fusain. In addition, the opaque matter is most abundant in the Phalen seam where the plant material in the coal has undergone great decay (Plate III, Fig. 3).

Mineral Matter

The source of the mineral matter in coal may be extraneous or indigenous. It may be of organic or inorganic origin. In the Phalen seam, calcite has been found, and the mineral is stained by iron in certain parts of the seam. Quartz, carbonates and gypsum have been determined microscopically. Pyrite is the most important mineral of economic interest in the seam, and will be discussed more fully later. Shaly matter is common in portions of the seam and is chiefly of a carbonaceous nature.

Fusain

One of the most interesting constituents of coal is fusain. It includes the more highly carbonized matter consisting of thin layers and lenticles of original plant tissues whose structure may or may not be retained. Under the microscope, the fusain, when cellular, has the appearance of a finely reticulated structure. Some of the most delicate plant structures are sometimes exquisitely preserved in the fusain.

The fusain of the Phalen seam is of two types microscopically.

1. Fusain with well defined cell structure (Plate II, Fig. 4).

2. Fusain composed of structureless, compact masses probably formed by the collapse of the cellular structures (Plate III, Fig. 3).

A few cases of gradation from anthraxylon to cellular fusain were observed. The sequence begins with anthraxylon, which gradually becomes cellular, then more opaque, and finally cellular fusain is formed.

Fusain, if present in any quantity, is believed to be deleterious to coking, but this is debatable. It is also believed to furnish most of the dust found in coal mines. In the Phalen seam the fusain forms about 2% of the coal.

Analyses

Analyses of anthraxylon, attritus and fusain were furnished through the courtesy of R. E. Gilmore, Superintendent of the Fuel Research Laboratories of the Department of Mines at Ottawa. The different components were carefully selected from the pillars, special care being taken to keep the specimens as pure as possible.

The proximate analyses indicate that fixed carbon is highest in anthraxylon and lowest in attritus. As would be expected, owing to the presence of spores, resins and cuticles, the volatile matter is highest in attritus and lowest in

TABLE I

ANALYSES OF COMPONENTS OF PHALEN COAL

—	Anthraxylon		Attritus		Fusain	
	As received	Dry	As received	Dry	As received	Dry
Proximate analyses						
Moisture, %	2.7	—	2.1	—	1.3	—
Ash, %	4.1	4.2	7.2	7.3	17.2	17.4
Volatile matter, %	29.3	30.1	32.6	33.3	22.5	22.8
Fixed carbon, %	63.9	65.7	58.1	59.4	59.0	59.8
Ultimate analyses						
Carbon, %	80.0	82.3	76.1	77.8	69.7	70.7
Hydrogen, %	5.0	4.8	5.1	5.0	3.0	2.9
Ash, %	4.1	4.2	7.2	7.3	17.2	17.4
Sulphur, %	1.3	1.3	2.3	2.3	1.1	1.1
Nitrogen, %	1.4	1.4	1.5	1.6	0.5	0.5
Oxygen, %	8.2	6.0	7.8	6.0	8.5	7.4
Other data						
Calories per gm. gross	7,860	8,080	7,560	7,725	6,205	6,290
B.t.u.'s per lb. gross	14,150	14,550	13,610	13,910	11,170	11,320
Carbon-hydrogen ratio	16.0	17.0	14.9	15.7	23.1	24.3
Fuel ratio	2.20		1.80		2.60	
Softening temperature of ash, ° F.	2000		2115		2680	
Specific gravity (apparent)						
Lumps	1.27		1.42		1.48	
Fines	1.26		1.23		1.29	
Coking properties	Good		Fair		Non-coking	

fusain. Since the anthraxylon is the purest component in coal, it contains the least ash. The attritus with its multivariant components has a high ash content. The fusain however has the highest ash content. The cellular spaces of the fusain were probably impregnated with mineral matter which, strangely, is not pyritiferous. Carbonates and silica probably compose most of the fusain ash. The attritus contains the highest percentage of sulphur.

The coking properties of the various components are interesting. It has been suggested that coals composed for the most part of woody constituents are the best coking coals. Anthraxylon, the component richest in woody matter, has the best coking properties, while fusain does not form coke at all. Singularly, the heating value is highest in the anthraxylon and least in the fusain.

Physiographic Conditions of Deposition of the Phalen Seam

A knowledge of the conditions under which the Phalen seam was laid down is of importance to the coal miner and geologist, not only from a scientific point of view, but for the information it may furnish regarding the more

economical methods of selective mining, upon which the future of the field depends. The exhaustion of the land workings and the growing dependence of the field on undersea mining has necessitated the adoption of more careful methods of extracting the coal.

As far as the writer is aware, no attempt has yet been made to interpret the physiographic conditions of deposition of an individual coal seam on the basis of the microstructure of the coal. Any features revealed by the microstructure which would indicate the more favorable localities for future mining development would be of value to the coal operators.

Hayes and Bell (6) state that flood-plain deposition in a progressively subsiding river valley was the mode of origin of the Sydney coal field. The coal-forming swamps were subject to different conditions and, as a result, coal of various types and qualities was deposited. It is quite possible that, throughout the whole coal field, streams were flowing into the swamps and contaminating the plant matter with various inorganic sediments. Conditions near the border or shore line of the swamp would be different from conditions existing in the central part of the swamp.

The Phalen seam was formed under uniform conditions in the central part of the basin. Towards the edge of the basin, impurities are more prevalent and a thinning and splitting takes place in the seam. This seam is particularly well suited to microscopic study as it is the best known of the various seams in the field.

Formation of the Phalen Seam as Interpreted by the Microscopic Study

The microscopic components of coal are found in different proportions throughout the Phalen seam. The distribution of these components is of interest in that it throws light on the manner of origin and mode of formation of this seam.

The microscopic study was confined solely to the Phalen coal in the central part of the field, in Lingan, Bridgeport and Glace Bay basins. The equivalents of the seam in the Morien basin to the west of Sydney Harbor were not examined microscopically. The 13 pillars of coal which were studied show a gradual change in microscopic character from Sydney Harbor to Cape Percy. The coals in the pillars can be divided into three groups (Fig. 2).

Group 1:— Coals characterized by high spore, high pyrite and low resin content. Pillars *A*, *B*, *C*, and *E* are of this type (Plate I, Figs. 2 and 3; Plate II, Figs. 1–4).

Group 2:— Coal intermediate to Groups 1 and 3. This coal is a moderate-spore, low-to-moderate pyrite and moderate-resin type, and is represented by Pillars *D*, *F* and *I* (Plate II, Figs. 5 and 6).

Group 3:— High-resin, high-pyrite, and low-spore coals. Pillars *G*, *H*, *J*, *K*, *L* and *M* belong to this group (Plate III, Figs. 1–4).

Group 1 (Pillars A, B, C and E)

These pillars are located in the Lingan and western part of the Glace Bay regions. The spore content, the most distinctive feature of this group, is indicative of open-water deposition. Whether the spores were carried into the quiet pools of open water in the centre of the swamp by wind and water action is a question of some moment. Probably both forces would act if open-water conditions obtained for a considerable period of time, and, if there were no outside source of introduced plant matter, a cannel coal would be formed. A coal that appears to have a small spore content may have as many spores as a high-spore coal throughout the attritus, but, owing to the preponderance of woody tissue, may seem to be deficient in spore content. The proportion of spores increases in the middle of the pillars, which corresponds to the most stable conditions in the swamp. In this section of the Phalen seam, additional plant material was added to the swamp, and the result was a banded coal with a high spore content. The coal from this part of the field has had a large sale in the Boston and New York markets because of its high gas content and the illuminating qualities of the gas, properties which are a result of the spore content.

Associated with the spores are clear translucent bands of anthraxylon and humain. This would indicate that the process of decay had not been very active. In fact, open water in swamps is known to be a remarkable preservative of woody tissue. The greater thickness of the coal seam in the neighborhood of Pillars *C* and *E* could be accounted for in this manner. More plant matter would have been deposited in the part of the swamp least subject to open-water conditions. The greater degree of putrefaction, however, would tend to concentrate the vegetable matter, and would produce a coal which would show evidence of great decay. Therefore, a section of the swamp with a small supply of plant matter, and subject to little decay, could deposit as much embryonic coal as a section with a large supply of plant matter, but under conditions of great decay.

Resinous bodies, whose presence is a criterion of conditions of decay, are not common, but tend to increase in quantity towards the east. Opaque matter, which is possibly a product of extreme putrefaction, is not present in any quantity. The absence of pyrite and shaly matter is an indication of the inaccessibility of this region to the contaminating waters carried into the swamp. Fusain is scattered throughout the pillars in small lenticles which increase in quantity at the top and bottom of the pillars. The cell structure is well preserved in the fusain. The coal of this group is believed to have been deposited under the quiescent open-water conditions of the central part of the swamp. The components are best preserved here, producing a pure type of banded bituminous coal which is featured by its high spore content.

Group 2 (Pillars D, F and I)

The pillars were extracted from the central and western parts of Glace Bay basin. The coal of this group is intermediate in type to Groups 1 and 3.

The spore content decreases, while the resinous bodies and the disseminated pyrite increase, especially at the top and bottom of the pillars. The humain is not as translucent as in Group 1, and opaque matter is beginning to be more noticeable.

Coal of Group 2 is an intermediate stage in the passage from the more open-water conditions of the central part of the swamp, and the shore-line conditions that existed in the vicinity of Cape Percy.

Group 3 (Pillars G, H, J, K, L and M)

The pillars of this group were extracted from the eastern part of the Glace Bay region. The microstructure of the coal of this group is in marked contrast with that of Group 1; the abundance of resinous bodies is a distinctive feature. The accumulation of these resinous bodies would indicate that conditions of great decay existed in this section of the swamp. The woody tissue and other bodies less resistant to putrefaction were decomposed, and left a concentrated mass of humain and resin. The resins increase at the top and bottom of the pillars and in the vicinity of Cape Percy.

The high pyrite content of the coal from this section is an important economic factor, because it lowers the value of the coal. Pyrite is so abundant in places that it is disseminated in the form of lenticles. It shows the same distribution as the resinous bodies. Mineral and shaly matter also increase in the vicinity of Cape Percy. Shaly lenses are found in pillars *K*, *L* and *M*, and are more abundant at the top and bottom of the pillars. The pyrite and shaly matter were deposited at the time the seam was formed, and a progressive increase of those substances as Cape Percy is approached would indicate that their source had been in this region.

With the increase in pyrite and resins, the spores decrease. In most cases spores are present in small numbers, but as a rule they are practically absent. The amount of spores deposited in coal of Group 3 would be much less in proportion to the amount of woody matter deposited than in Group 1. Even the highly resistant spores show evidence of greater putrefaction in this part of the seam.

The anthraxylon is not of the clear translucent type found in Group 1. Pyrite bodies and opaque matter constitute impurities in it. Anthraxylon lenticles or bands are most abundant in this group, a result of the deposition of the more massive plant structures that grew near the edges of the swamp.

The humain of this group is quite different from the clear, structureless and translucent humain of Group 1. It is composed of numerous brecciated and cemented fragments of woody tissue possessing structural evidences of great decay. Opaque matter is intimately mixed in greatly increased proportions with the humain. With the increase of opaque matter, there is a similar increase in fusain. The fusain, however, is still more abundant at the top and bottom of the pillars. The well defined cell structure, characteristic of the fusain in the western part of the field, is replaced by a type in which the cell structure has been destroyed, with the formation of a black, structureless, opaque mass.

The degree of putrefaction shown by the various constituents of the coal would indicate that deposition had taken place near the edge of the swamp where conditions of decay were greatest. The presence of a stream that entered the swamp in the vicinity of Cape Percy would also be evidence of shore-line conditions. This stream would not only provide a source for the pyrite and other impurities in the coal, but would be an effective aerating agent, and would produce conditions favorable for extreme decay.

Certain constituents in the coal seam, among which are resins, opaque matter, fusain, shaly matter and pyrite, are more common at the top and bottom of the seam. The transgressive nature of the swamp was evidenced by the existence of a moving shore line, which resulted in a difference in the microscopic constituents of the coal at the top and bottom of the seam. During the period of greatest stability, when the Phalen swamp occupied its greatest extent, more spores were deposited in the central part of the basin. As a result, the coal in the middle section is richer in spores than that at the top and bottom of the seam.

Analysis of the Pillars

A series of complete proximate analyses of samples from the top to the bottom of each of the 13 pillars was made in the Fuel Research Laboratories of the Mines Branch at Ottawa. The average analysis of every pillar was plotted for the fixed carbon, volatile matter, ash and pyrite content. The divisions or groups of the pillars based on microscopic features were retained for a comparison of their chemical differences (Fig. 5).

The proportion of fixed carbon is quite uniform in Groups 1 and 2, not varying more than 4%, except in Pillar 1 where the coal is approaching Group 3 in type. Group 3 shows a progressive decrease in fixed carbon to the east.

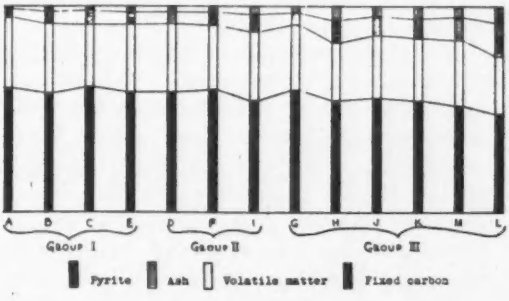


FIG. 5. *Variation in the average proximate analyses of coal pillars from the Phalen seam.*

The volatile matter does not show great variation because both resinous and spore coals are high-volatile coals. It is present in smaller quantities in the pillars that contain a high percentage of mineral matter and ash.

The ash content, as is to be expected, is highest in Group 3 and lowest in Group 1. The shaly matter and pyrite are responsible for the ash.

The sulphur content, as has already been observed, is highest in Group 3 and lowest in Group 1. Both the pyrite and ash content of the coal increase as Cape Percy is approached.

A chemical variation, as well as a change in the microstructure, is evident in the seam. The differences in the chemical nature of the coal are due mainly to the influx of impurities into the swamp in the vicinity of Cape Percy.

The pyrite content has been contoured (Fig. 2); its increase towards Cape Percy is a striking feature.

Gray (4, pp. 62–71) has propounded an interesting theory to explain the high sulphur and ash content of the coal in the vicinity of Cape Percy. He suggests that in this locality there was a spur of high ground projecting into the basin of deposition, thus causing a shallowing of the swamp and a deterioration of the coal in that part of the seam.

If this elevation existed during the period of coal formation, a definite thinning of the strata should be observed in the vicinity of Cape Percy. With this in mind, the stratigraphic intervals * between the Phalen and the Backpit, Emery, and Gardiner seams were calculated. These intervals were plotted throughout the field (Fig. 1). They show that there is no pronounced thinning of strata in the vicinity of Cape Percy. On the contrary, the stratigraphic intervals between the seams are greater in this part of the field than to the west. Therefore, no elevation could have existed in the vicinity of Cape Percy.

Future Mining Possibilities

It seems probable that the coal seams of the Sydney field extend so far out to sea that it will be impossible to mine them completely. As the Phalen seam dips gently seaward, and no serious folding movements have taken place, conditions appear very favorable for extensive mining development under the sea.

The mechanical difficulties in the mining of the undersea coal are not the only features to be considered. In operations of such magnitude, a deterioration in the quality of the coal would be disastrous. It is necessary to determine where the best and purest coal is located for future mining operations.

The purer coal of the central part of the basin is found to the north and west of the Bridgeport anticline, thus favoring future mining operations in this neighborhood. The high-sulphur and high-ash coals in the eastern part of the field should decrease progressively seaward as the source of the original impurities would have receded. Open-water conditions of deposition were probably more prevalent in this region; hence a purer type of coal was produced.

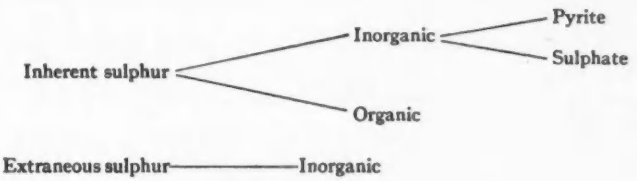
There is, therefore, every reason to believe that favorable conditions exist for an extensive undersea development of the Sydney coal field.

Sulphur in Coal

Sulphur is an important and universal constituent of coal, and in small proportions has little effect on it. Coals of a high sulphur content, however, have all their valuable properties modified, resulting in a coal of an inferior quality.

* *Estimated figures furnished by W. A. Bell, Geol. Surv. Can., 1932.*

Sulphur is found in several different forms in coal. It may originate either contemporaneously with, or later than, the coal. The former type may be termed inherent, while the latter may be called extraneous. The inherent sulphur can be of organic or inorganic origin, while the extraneous sulphur is always inorganic.



Inherent Sulphur

Inorganic Sulphur

Pyrite. The chief sulphur-bearing mineral in coal is pyrite. It is found in several different forms (1).

1. In bands parallel to the bedding.
2. In flat lenses.
3. In balls and rounded masses.
4. As disseminated bodies occurring in fine microscopic particles.

Sulphate sulphur. Sulphates are not abundant in coal; of these, gypsum is the commonest. Sulphates may be carried into a swamp and deposited there, or they may form as an oxidation product of pyrite.

Organic sulphur. This type of sulphur is mainly amicroscopic. According to Thiessen (9), bodies of disseminated pyrite are sometimes of organic origin.

Extraneous Sulphur

This type of sulphur, chiefly in the form of pyrite, is introduced after the coal has been formed. It crosses the bedding and penetrates the joints and fractures in the coal, forming plates and sheets of pyrite. The roof of the coal seam containing extraneous pyrite should be closely examined in order to determine the extent of fracturing. Highly fractured roofs would permit the penetration from above of sulphide-bearing solutions and deposition of sulphides in the seam. An undisturbed roof would indicate a coal relatively free from this type of pyrite.

Origin of Sulphur in Coal

The sulphur in coal may be formed by the infiltration of marine waters or sulphur solutions into the swamp. Bacterial and plant sulphur are additional sources.

Marine Conditions

The high percentage of sulphur in certain coal basins is attributed to marine submergence of the coal-forming peat deposits. A relation between

high-sulphur coals and roofs of marine origin containing remains of marine invertebrates has been observed (2). Whether the iron and sulphur salts in sea water are present in proportions sufficient to produce pyrite when precipitated by the decaying organic matter is open to question. It is noteworthy that most of the high-sulphur peats are salt-marsh peats.

Sulphur Solutions

Solutions bearing sulphur and iron may be carried into a swamp and the sulphur and iron precipitated. The iron may come from the iron-containing minerals of the surrounding land, while gypsum beds may provide an adequate source for the sulphur.

According to Harder (5), during the process of putrefaction of the vegetable debris, carbon dioxide and marsh gas are evolved. The marsh gas attacks any calcium sulphate with the evolution of hydrogen sulphide. Marsh gas also reduces the iron salts of the vegetable debris producing ferrous sulphide. Coal-forming material containing a local excess of ferrous sulphide would produce pyrite bands (5, pp. 74-75, 82-84).

Iron and sulphur solutions are precipitated by organic products of decay. In fact, it is possible that in the swamp the iron may be in the form of soluble salts of certain humic acids. Iron carbonate and limonite are often found in peat bogs which may form pyrite by the decomposition of calcium or magnesium sulphate with the liberation and removal of calcium carbonate. The iron carried into the swamp may be precipitated directly as a black colloidal hydrate which, in the presence of hydrogen sulphide under reducing conditions, is changed to hydrated ferrous monosulphide, and this in turn is changed to pyrite (5, pp. 74–75, 82–84). The rate at which these solutions enter and are precipitated in the swamp varies greatly, producing layers of high and of low sulphur content.

Bacterial Sulphur

Sulphur and iron bacteria are well known in nature. These organisms obtain vital energy from the sulphur and iron compounds.

Certain sulphur bacteria produce hydrogen sulphide (5, pp. 74–75, 82–84) by the decomposition of the sulphur-bearing proteins. Other sulphur bacteria have the ability to reduce sulphates. In the presence of decaying organic matter they take oxygen from the sulphates and form sulphides. Another type acts directly on free sulphur to form hydrogen sulphide which acts upon ferrous salts in solution.

Iron bacteria are also active in breaking down iron salts in solution. They store ferric oxide in their cell walls and under favorable conditions produce deposits of bog-iron ore. An iron oxide ball built up by these bacteria, if lying in coal, may under suitable conditions be changed into pyrite.

Thiessen (9, pp. 919–923) has recorded the presence of pyrite-producing bacteria in peat. Some of the microscopic disseminated pyrite found in higher-rank coals is possibly of this origin.

Plant Sulphur

Sulphur in plants consists of two types, protein sulphur and non-protein sulphur. The sulphur in the proteins is set free during putrefaction. There is more than enough sulphur in plant material (9, pp. 919–923) to supply all the sulphur in coal, considering that the sulphur content remains constant and the plant matter decreases in volume in the process of coalification.

Plant sulphur in coal is amicroscopic and can be determined only by chemical tests. Two types of plant sulphur are distinguished in coal, humic sulphur and resinic sulphur.

Sulphur in the Phalen Seam

Types and Distribution

The sulphur content is an important feature of the Phalen seam. The sulphur percentage is so high in certain sections that it has a deleterious effect on the coal. The sulphur is found in organic and inorganic forms. The organic sulphur is of course amicroscopic, and is determined only chemically. The inorganic sulphur is mainly pyrite.

The pyrite ranges from one micron to macroscopic dimensions. It is distinctly of the disseminated type, and in places it becomes so abundant that lenticles of disseminated pyrite are formed. The pyrite is in the form of small, oval, opaque bodies which show the characteristic brassy yellow of pyrite in reflected light. The pyrite is most abundant in the attritus although pyrite bodies are also found in the anthraxylon (Plate III, Fig. 4). No pyrite was detected in the fusain.

The chemical analyses and the microscopic examination of the Phalen seam reveal a variation in the pyrite content of the seam both horizontally and vertically. Pyrite increases in the top and bottom of the seam, and this relation holds throughout its whole extent. The gradation coincides directly with the variation in microstructure of the seam. A high-spore coal is essentially a coal low in pyrite (Group 1). An increase in pyrite is noticed in the section of the seam that has a moderate spore and resin content (Group 2). The resin coals are the ones which are richest in the percentage of pyrite (Group 3 (Fig. 2)). In Group 3, pyrite is very evident, producing a high percentage of sulphur in the coal. The pyrite content of the coal progressively increases as Cape Percy is approached.

Analyses were made in the Fuel Research Laboratories at Ottawa for the types of sulphur found in the Phalen seam. Pyritic sulphur, organic sulphur and sulphate sulphur were determined. Sulphur in the low-sulphur coal is mainly organic, while in the high-sulphur coal it is found in the form of pyrite. The sulphate content, for the most part gypsum, is negligible.

Origin

The Phalen seam was formed under strictly terrestrial conditions. Therefore infiltration of marine waters would not be a factor in the formation of

the pyrite in the seam. Bacterial and plant sulphur, although contributing to the total sulphur, could not account for the high pyrite content and its peculiar distribution in the seam.

That sulphate and iron solutions entered the swamp and formed pyrite, offers the most satisfactory explanation for the origin of the pyrite. Iron, in various forms, and gypsum deposits are found in various localities throughout the Sydney coal field.

The pyrite distribution would indicate that the source of the pyrite was a river carrying sulphate- and iron-bearing waters which entered the swamp in the vicinity of Cape Percy. The stream came into the swamp from the westward rather than the eastward side of Cape Percy. The pyrite content in the Phalen seam decreases to the west. In the Morien basin the sulphur is highest in that part of the Gowrie seam nearest to Cape Percy. Evidence obtained with the microscope tends to confirm the conclusion that there was an influx of sulphur- and iron-bearing waters in the vicinity of Cape Percy, that is to say, the higher sulphur content of the Phalen seam in this neighborhood is a strictly local condition associated with approach to the edge of the original coal swamp.

There was apparently a greater influx of the sulphur- and iron-bearing waters when the swamp was first formed, which decreased when conditions of swamp formation were more uniform and the swamp had reached its maximum extent. In the closing stages of swamp formation, when one set of conditions was changing to another, there was another increase in the influx of these waters. A coal high in pyrite at the top and bottom of the seam was thus produced.

The coal nearest the source of the sulphur and iron is naturally highest in pyrite content. The percentage of pyrite is highest where decay had been most active in the swamp. The organic products of decay were effective precipitating agents of the iron- and sulphur-bearing waters.

There is, therefore, no reason to expect an increase in the proportion of pyrite as the seam is worked progressively seaward. On the contrary, the pyrite content should decrease. The best type of coal for future development would be found to the west of the coal comprised in Group 3.

Spore Correlation in the Sydney Coal Field

Correlation of the strata and coal seams in the Sydney field is fraught with difficulties. No reliable correlation of the coal seams can be based on a comparison of thickness and lithology of the individual beds, owing to the nature of flood-plain deposition. The subdivision of the field by structural and erosional features, as well as by independent mining operations, has given rise to different names for the same seam throughout the field. A knowledge of the extent of the various seams in the field is of great importance to the mining companies as a guide for future mining operations.

The recently developed method of correlating coal seams by means of the spore types contained in the coal and by their distribution in the seams is of

interest. The method has already proved of value in coal areas where correlation by stratigraphic and ordinary paleontologic methods is difficult. As a preliminary step, the spores in the Phalen seam were examined and classified into various groups and types. As yet, a microscopic examination of the spores in the other seams of the field has not been initiated. The writer hopes that the following account will furnish a basis for a future investigation which has great possibilities.

In order that a spore may be of value in correlation, it must have a definite physical appearance by means of which it may be easily recognized. In other words, this type of correlation is based on the theory that there was a change in the type of plant in the intervals between the depositions of the various coal beds. The change would manifest itself in differences in types of spores.

In England it was found (7, p. 8) that no particular type of spore would serve to identify a seam by its presence. The varying concentration of several types of spores in different horizons in the seam, however, proved characteristic. The statistical method of fossil distribution, common in palaeontology, was used as the main basis for the correlation of English coal seams.

The method of spore counts may be satisfactory in seams where uniform conditions prevailed over great areas, but where conditions changed, this method is not adequate. In the Sydney field, conditions were not uniform,

TABLE II

MEGASPORES IN THE PHALEN SEAM (FIG. 3)

Spore	Figure	Size, μ	Description	Distribution
1	A	110–350 Average 170	Smooth-walled	Most abundant megaspore. Chiefly in Group 1
2	B	250–300	Smooth-walled with pointed ends	Abundant in Groups 1 and 2
3	C	200–500	Has a striated tuft-like appendage	Present only in Groups 1 and 2
4	D	100–500	Very slender, smooth-walled	Practically confined to Group 1
5	E	220–270	Smooth, double-walled	Confined to Group 1
6	F	240–340	Thick, with tubercles	Not common, and confined to Group 1
7	G	200	Serrated	Rare
8	H	220	Squat, bulky	Unique
9	I	150	Bulky, with square tubercles	Occurs in Pillar K

and it has already been pointed out that the spore content of the Phalen seam varies greatly. In the Lingan basin, the coal contains enormous numbers of spores, chiefly microspores. To the east, a progressive decrease in the quantity of spores has been noted, and in places they may be practically absent. Graphical methods of comparing the quantity of spores in different parts of the field would thus be valueless. The only remaining satisfactory method would be the detection of singularly characteristic spores in the coal bed. Spores of this type have been found in the United States.

The spores in the Phalen seam are as a rule smaller than the types of spores found in the English and American coals. The microspores range in length from 6 to 40 μ, and megaspores from 100 to 500 μ.

TABLE III

MICROSPORES IN THE PHALEN SEAM (FIG. 4)

Spore	Figure	Size, μ	Description	Distribution
1	A	Average, 14	Smooth-walled	Commonest microspore. Found often in layers. Most abundant in Group 1
2	B	23–40	Nodular	Abundant in Groups 1 and 2 and uncommon in Group 3
3	C	Average, 7	Small, thick, with smooth and nodular varieties	Present mainly in Group 1
4	D	Average, 13	Serrated	Present only in Groups 1 and 2
5	E	Average, 18	Pointed ends with smooth, nodular and serrated varieties	Most abundant in Group 1 and least in Group 3
6	F	Average, 6	Very slender, small	Predominant in Group 2
7	G	Average, 13	Salver-shaped	Most abundant in Group 1, decreasing progressively eastward
8	H	Average, 26	Thin-walled, spinose	Uncommon and found chiefly in Group 3
9	I	Average, 28	Stout, with fleshy tubercles	Found mainly in Group 3. Uncommon
10	J	Average, 13	Notched	Uncommon and found only in Group 3
11	K	Average, 11	Smooth, double-walled	Sparingly in Group 1

Acknowledgments

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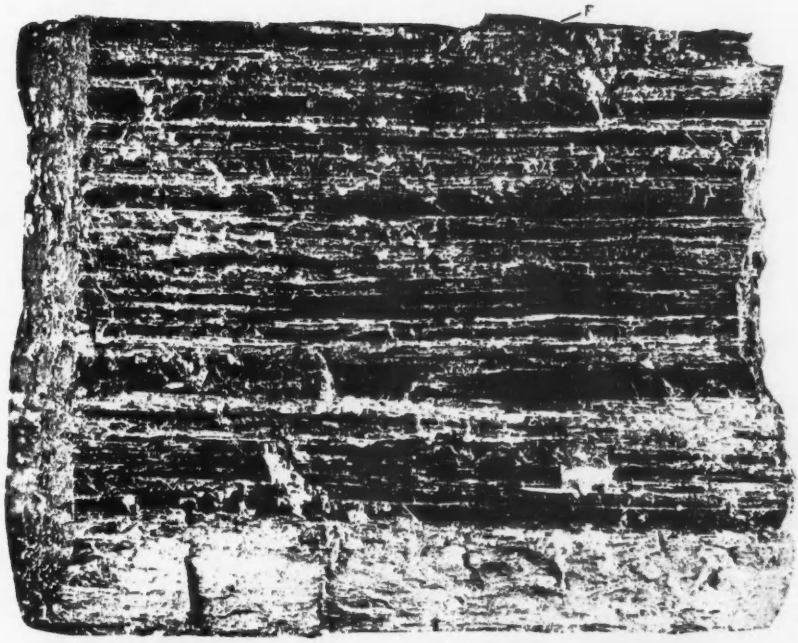


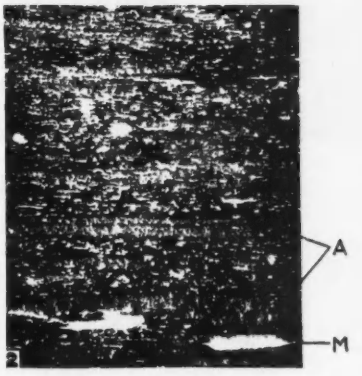


FIG. 1. *Coal from Pillar M, Phalen seam. The banded character of the anthraxylon, or bright coal, and the attritus, or dull coal, is clearly shown. A lenticle of fusain (F) is found at the top. A layer of carbonaceous shale can be seen at the bottom of the specimen.* × ½. FIG. 2. *Pillar B, Section 10. Microspores are abundant. The megaspores (M) at the bottom are of the sharp-pointed variety described as Type 2. A few thin bands of anthraxylon (A) are clearly distinguished.* × *31.* FIG. 3. *Pillar E, Section 22. The layer of megaspores is composed of the smooth-walled Type 1.* × *61.*

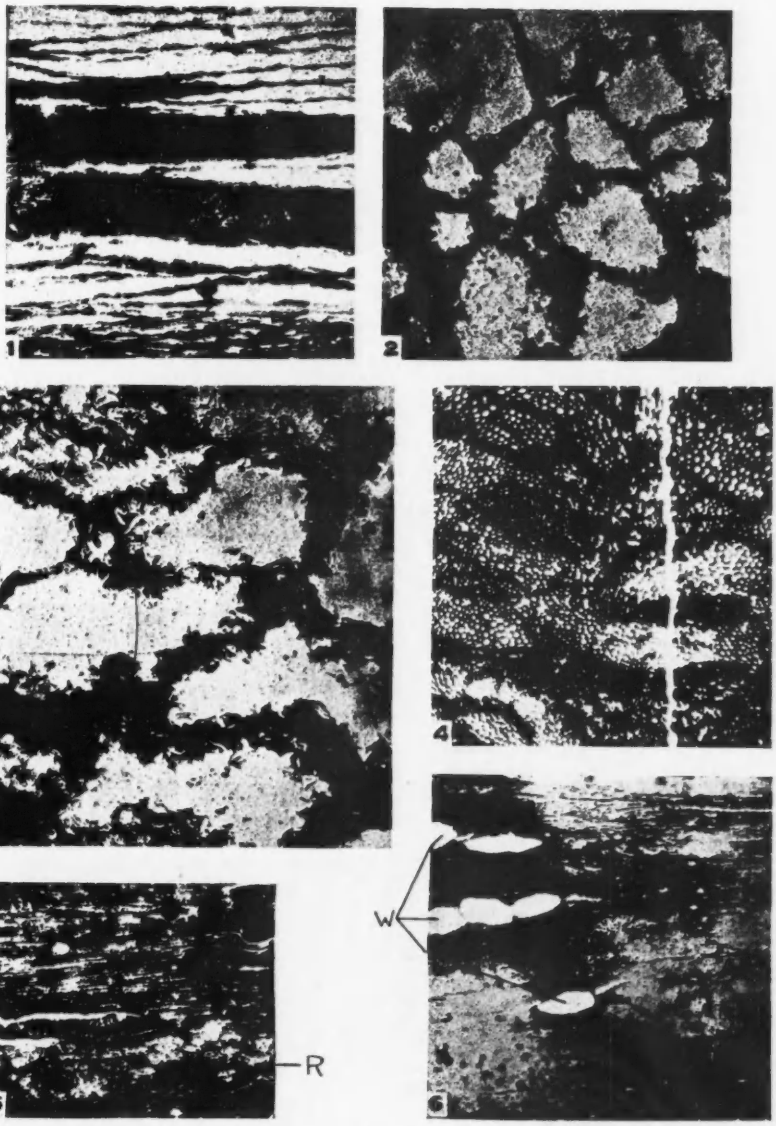
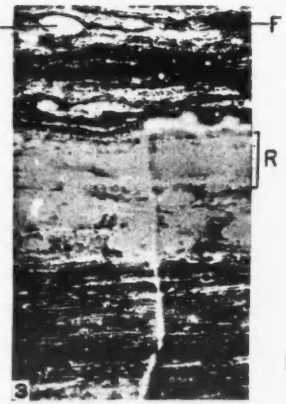


FIG. 1. *Pillar E, Section 18. Microspores belonging to Type 1 compose the layers. The layers may be remains of sporangia flattened by pressure. A forking anthraxylon lenticle separates some of the layers.* × *33.* FIG. 2. *Pillar E, Section 18. Possibly a horizontal section of microsporangia as shown in Fig. 1. The circular outline of the individual microspores can be distinguished in places.* × *29.* FIG. 3. *Pillar E. Section 18. Same section as Fig. 2 only more highly magnified. In places the tetrad markings of the microspores can be distinguished. The arrangement of the spores suggests that these are the remains of sporangia.* × *117.* FIG. 4. *Pillar E, Section 4. Fusain showing well-preserved cell structure. The cellular spaces are empty.* × *33.* FIG. 5. *Pillar D, Section 5. The attritus is composed of relatively translucent groundmass with some opaque matter. A layer of resins (R) indicates a stage of extreme putrefaction. A few microspores and cuticles are scattered throughout the attritus.* × *33.* FIG. 6. *Pillar I, Section 11. Microspores are practically absent. Resinous bodies are abundant but are not clearly shown. Waxes (W) of a lemon-yellow color are distinctive. Opaque matter is fairly common.* × *73.*



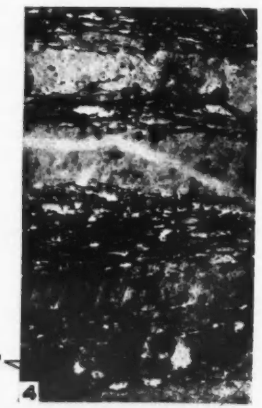


FIG. 1. *Pillar G, Section 5. Microspores are practically absent. The groundmass is composed of minutely comminuted fragments of woody tissue or humain with some opaque matter. Structureless fusain is found in lenticles. Resinous bodies are found throughout the section.* × *66.* FIG. 2. *Pillar G, Section 11. Opaque matter and humain make up most of the attritus. A cuticle (C) with a serrated border is shown. At the top of the section several large globules of resins (R) are seen.* × *33.* FIG. 3. *Pillar M, Section 18. The coal is of an impure nature. Opaque matter and fusain are present in large proportions. The attritus apparently was formed under conditions of great decay. Resins (R) are abundant.* × *33.* FIG. 4. *Pillar K, Section 11. Disseminated pyrite (P) is shown to be mainly confined to the attritus.*

PLATE III

investigation have proved of great assistance. He is indebted to Dr. W. A. Bell of the Geological Survey of Canada, who furnished him with information and notes, as yet unpublished, on the general geology of the field. Further, an expression of sincere thanks is due to Mr. R. E. Gilmore and Mr. J. H. H. Nicolls of the Fuel Research Laboratories, Ottawa, for helpful co-operation and for the analyses presented in this work. Finally, the constructive criticism of Mr. F. E. Lathe, Director, Division of Research Information, National Research Council of Canada, was of great value in the preparation of the manuscript for publication.

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THE RESIN OF WHEAT STRAW[1]

By Léo Marion[2]

Abstract

A study of the resin extracted from wheat straw by methanol-benzene (50–50 by volume) has shown that about one-quarter of it (or 1.5% of the straw) consists of a mixture comprising an oil, free fatty acids, a wax and a small quantity of an essential oil. On saponification, the oil yields sitosterol and a mixture of fatty acids (palmitic, stearic, oleic, linolic and lignoceric acids were identified), while the wax yields ceryl alcohol, sitosterol, palmitic and cerotic acids.

The remainder of the resin (4.7% of the straw) consists mostly of lignin, but contains in addition small quantities of phytosterolin, sterol ester, i-inositol and potassium nitrate.

A previous paper by the writer (10) dealt with the isolation of lignin from the straws of wheat and other cereals. In the isolation of lignin from wheat straw, the latter was first extracted with a mixture of methanol and benzene, the extract yielding a sticky, semifluid mass generally designated as resin. It represented 6.22%* of the weight of dry straw. Comparatively little is known concerning this resin beyond the fact that it comprises a material of waxy consistency (5).

As early as 1869 Radziszewski (14) isolated substances of waxy consistency from cereal straws but did not investigate them. Later König (8, 9), working with rye straw and oat straw, stated that he had effected a separation of the wax from the fat which he considered as consisting of tristearin, tripalmitin, and triolein together with a hydrocarbon, ceroten.

The petroleum ether extract of wheat straw contains, besides glycerides, an appreciable proportion of wax. Concerning this wax, Heuser (5), in a cursory study, mentioned that it is light yellow and that it forms 1.18% of the weight of dry straw. He had not, however, effected any separation of the fat and wax, and was dealing with a mixture.

It is known that the oils extracted from the various parts of the wheat kernel such as the germ, inner endosperm and the bran, differ markedly from one another (2), and the dissimilarity has been found to extend to the oil present in the stalk or straw which is identical with none of the former.

About one-quarter of the resin, or 1.5% of the weight of straw, was found by the author to be extractable with petroleum ether and consists of a mixture of a fat or oil, and a wax, together with a small quantity of essential oil. The oil, when separated from the wax, has the constants recorded in Table I. For purposes of comparison the constants of wheat-germ oil, determined by Jamieson and Baughman (7), are also given in the same table.

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Contribution from the Division of Chemistry, National Research Laboratories, Ottawa, Canada.

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**This was reported previously (10) as 11.4%. It has now been found that extraction with methanol-benzene for 24 hr. removed 6.22%, while a second and a third extraction, each of 24 hr., with methanol removed a further 3.5% and 0.8%, totalling 10.5%. The methanol extracts were found to be largely soluble in water. They were not further investigated.*

Free acids are present in the straw oil in rather large quantities and consist of palmitic, stearic and lignoceric acids. No resin acids whatsoever are present. The total acids obtained after saponification amount to 42.0% of the fat. They include oleic, linolic, stearic, palmitic and lignoceric acids together with a small quantity of a waxy substance which is insoluble in alcohol. In the unsaponifiable, which is present in considerable quantity, no alcohol other than sitosterol, m.p. 137° C,* could be detected. Since Anderson, Shriner and Burr (1) have shown the wheat-germ sterol to be a mixture, the present sterol is also probably heterogeneous: it was not further examined, although it was purified through its acetate.

TABLE I

PHYSICAL AND CHEMICAL CONSTANTS OF WHEAT-STRAW AND WHEAT-GERM OILS

	Wheat-straw oil	Wheat-germ oil
Refractive index	(26° C.) 1.4952	(20° C.) 1.4762
Acid value	35.6	7.6
Saponification value	86.6	186.5
Iodine value	(Waller) 57.5	(Hanus) 125.6
Reichert-Meissl value	1.07	0.2
Polenske number	1.35	0.35
Unsaponifiable, %	49.6	4.7
Saturated acids, %	12.1	13.3
Unsaturated acids, %	29.9	75.3
Insoluble in alc., %	3.2	—

The alcohol-insoluble fraction after purification and distillation consisted of small shining flakes, m.p. 70° C. On hydrolysis it produced cerotic acid, and probably consists of impure ceryl cerotate. Both the analytical figures and the production of cerotic acid on hydrolysis point to that conclusion, although its insolubility in alcohol does not agree with the recorded properties of that ester.

The wax, which is a soft, white solid (saponification no. 64.3; iodine value, 25.8), yielded ceryl alcohol (acetate m.p. 64° C.), a mixture of palmitic and cerotic acids and sitosterol, m.p. 137° C. Cerotic acid has recently been reported in rye straw (13) without, however, any study of the wax having been made. The phosphorus content of the wax amounts to 0.042% P_2O_5. Although this cannot be taken as an estimation of the phosphatides present, it may possibly be assumed to indicate the presence of a trace of the latter in wheat straw.

In addition to the foregoing substances the extract seems to contain a sterol ester. It was not possible to isolate the ester in a pure condition but, after saponification, a crystalline sterol which yielded an acetate, m.p. 131° C. was isolated. The presence of a sitosterol ester in wheat-germ oil has been reported by Ball (2), although the evidence is rather circumstantial. Ball, who also experienced a great deal of difficulty in purifying his product, failed to isolate the sterol in crystalline form and detected it by colorimetric tests only. In the present instance, the isolation and characterization of the sterol

**All melting points are corrected.*

seem to indicate the presence of a sterol ester in wheat straw and might perhaps support Ball's evidence for the presence of such a compound in wheat-germ oil.

A phytosterolin, characterized through its acetate, m.p. 159° C., and by Whitby's color test (15), has also been isolated from the extract. It has been further characterized by admixture with the sterolin from *Adlumia fungosa* (11), which failed to depress its melting point, and by hydrolysis. A sterolin has recently been isolated by Nakamura and Ichiba (12) from the wheat embryo, and the properties recorded by them are in agreement with the foregoing. The two substances have identical melting points and the sterols obtained on hydrolysis in each case melt at 137° C. Nakamura and Ichiba prepared an acetate which melts at 167° C., whereas that obtained in this case melts at 159° C., but this may be owing to the presence of a trace of impurity, the quantity on hand being too small to permit further purification. A mixture of this acetate with that obtained from the sterolin of *A. fungosa* melts at 157° C.

The resin contains a large proportion of lignin which is readily obtained after separation of the fat and wax. Some of the lignin isolated is no longer soluble in methanol or benzene. Since an appreciable amount of lignin is generally dissolved from straw by an organic solvent only in the presence of a catalyst, the removal of these quantities of lignin by methanol-benzene would tend to show the presence in straw of a small proportion of lignin which is not as firmly held as the major part of it. It has been shown that alcohol-benzene will remove small quantities of lignin even from ground wood (6), although lignin is normally removed from straw by much milder methods than those required to remove it from wood. Analogous cases of readily extractable lignin are repeatedly found in the literature.

Potassium nitrate (3%) and a small amount of *i*-inositol, m.p. 224° C., have also been found to occur in the wheat-straw extract.

Experimental

Wheat straw, ground in a Wiley mill, was thoroughly extracted with a mixture of methanol and benzene (50% by volume). This treatment removes 6.22% of the weight of the straw and yields an extract which, after removal of the solvent, consists of a dark brown, soft, sticky resin having an iodine absorption value of 28.6. Approximately 0.5 kg. of resin was used for the present work. With the aid of methanol the resin was mixed with shredded asbestos and the methanol removed on the steam bath. The hard cake produced was crushed and extracted in Soxhlets successively with petroleum ether, ether, chloroform, ethyl acetate, and methanol, the residue being then extracted in a flask with water and dilute sodium hydroxide.

Petroleum Ether Extract

After removal of the solvent this extract amounted to 133 gm. and consisted of a soft, resinous solid. It was suspended in water and distilled for five hours in a strong current of steam. The aqueous distillate, through

which were scattered small globules of oil, was shaken with several portions of ether. The extract, after drying over anhydrous sodium sulphate and removal of the solvent, yielded a small quantity of a light-brown oil which, when distilled under diminished pressure (b.p. 75–120°C./4 mm.), was obtained as a clear yellowish oil having a refractive index (22.5° C.) of 1.4862.

Isolation of Free Fatty Acids

The extract which had been steam-distilled consisted of a mixture of fat, wax and free acids. It was collected in ether and shaken with several portions of 5% sodium bicarbonate solution. The combined sodium bicarbonate solutions were washed with ether and acidified, the separated acids being collected in ether. The solution was dried over calcium chloride and the ether was evaporated. A thick oil was left in appreciable quantity. The relatively large proportion of free acids was indicated by the high acid number of the fat (35.6). The oil was esterified with methanolic sulphuric acid by the preferential method of Wolff and Scholze (16) which is known to esterify the fatty acids while it leaves the resin acids in the free state. No acidic residue having been left in this case it can be concluded that resin acids are absent and that the free acids belong entirely to the fatty series.

The methyl esters of the acids occurring in the free state, when subjected to distillation under diminished pressure, yielded three fractions: Fraction 1 consisted of an oil (b.p. 140–147° C./3.5 mm.), which crystallized on cooling and melted at 23–24° C.; when saponified with alcoholic potash this ester yielded an acid which was recrystallized from a mixture of alcohol and ethyl acetate, m.p. 62° C., and was found to be identical with palmitic acid. Fraction 2 consisted of a slightly yellow oil (b.p. 165–169° C./3 mm.) which, on saponification, yielded stearic acid crystallizing from alcohol at 0° C. in small white masses. Fraction 3 was crystalline and, on saponification, produced lignoceric acid which was recrystallized several times from alcohol, m.p. 73–74° C. Equivalent: calcd. for $C_{24}H_{48}O_2$, 368; found, 375.

In order to determine the constants of the oil and determine the unsaturated acids a fresh lot was prepared by extracting wheat straw with light petroleum ether. The solution was concentrated, treated with charcoal and filtered. The rest of the solvent was removed in an atmosphere of carbon dioxide and the residue dissolved in sufficient ether to make a 25% solution. A small quantity of a white insoluble substance was filtered and washed with ether; it amounted to 7.4% of the petroleum ether extract. To the combined filtrate and washings was added twice its volume of warm acetone which caused the precipitation of the wax. The well stirred mixture was kept in the ice chest overnight, then filtered and washed with a small volume of ether-acetone (1–2 vol.). The filtrate, after the solvent was distilled off in an atmosphere of carbon dioxide, yielded 45.4% of a yellow oil. The white granular solid on the filter contained the wax and any phosphatides; it amounted to 47.2%.

Saponification of the Glyceride

After the determination of the various constants which are given in Table I, the bulk of the oil was saponified with alcoholic potash and separated into unsaponifiable and acidic portions.

Unsaponifiable Fraction

Quantitatively, the unsaponifiable matter was determined on a sample of the oil by the Kerr-Sorber method as modified by Hertwig, *et al.* (4). The main bulk of the unsaponifiable matter combined with that obtained in the quantitative determination was boiled with alcohol, and the crystalline product recrystallized from that solvent several times. No fatty alcoholic constituent was present, and the sterol, purified by the preparation of its acetate followed by hydrolysis, was obtained as glistening flakes, m.p. 137° C.

Isolation of the Fatty Acids

The acids, separated from the unsaponifiable matter, amounted to 42.0% of the oil. They were dissolved in alcohol, filtered from a small amount of insoluble (3.2%) which will be referred to further on, and separated into saturated (12.1%) and unsaturated acids (29.9%) by the lead-soap-alcohol method (3, p. 376). The percentages given are all based on the amount of fat. From the saturated acids palmitic, m.p. 62° C., and stearic acids, m.p. 68° C., were obtained by fractional crystallization, the former being present in appreciably larger quantity than the latter. On oxidation with cold, dilute permanganate by Lapworth and Mottram's method the unsaturated fraction yielded sativic acid, m.p. 173.5° C., and dihydroxystearic acid, m.p. 127° C., proving the presence of linolic and oleic acids.

A sample of wax (0.6445 gm.) was digested with concentrated nitric acid and its phosphorus content determined by converting into ammonium phosphomolybdate, dissolving in standard alkali and titrating the excess. The wax contains 0.042% P_2O_5.

The very thick, oily substance which had been separated from the fatty acids through its insolubility in alcohol was found to be soluble in ether, dioxane and benzene, from which solvents it separated on addition of alcohol, in a semicrystalline state. After repeated separations from dioxane-alcohol, the substance was mixed with methanolic sulphuric acid, enough benzene added to bring about complete solution, and the solution boiled on the steam bath for three hours. This treatment seemed to effect the removal of some impurity and the substance was then fractionated under reduced pressure. Fraction 1, which was a colorless oil, b.p. 240° C./3.5 mm., crystallized from alcohol in very small plates, m.p. 60–61° C., which could not be further identified owing to paucity of material. Fraction 2, a white crystalline substance, was recrystallized from ethyl acetate from which, by the cautious addition of alcohol, it separated as white flakes, m.p. 70° C. Calcd. for $C_{58}H_{104}O_3$: C, 82.02; H, 13.78%. Found: C, 83.33, 83.68; H, 14.28, 14.28%. When saponified with alcoholic potash in the presence of some

benzene, this substance yielded a crystalline acid, m.p. 79–80° C. Calcd. for $C_{26}H_{52}O_2$: C, 78.70; H, 13.22%. M.W. 396. Found: C, 78.21, 78.41; H, 13.07, 13.00%. M.W. (Rast) 348, 348. The analytical figures obtained for the substance and the acid derived from it are, therefore, in fair agreement with those calculated for ceryl cerotate and cerotic acid.

Saponification of the Wax

The wax, as already mentioned, amounted to 47.2% of the petroleum ether extract, and consisted of a soft white solid. It was saponified by refluxing with sodium alcoholate for one hour. To the solution, cooled to about 70° C., sufficient water at 50–60° C. was added to make the concentration of alcohol approximately 85% and the resulting solution was extracted several times with ligroin (60–90° C.). After washing the ligroin extract with four portions of 85% alcohol, and distilling the solvent under slightly diminished pressure, the residue (48% of the wax) was dissolved in boiling acetone and the solution was filtered while hot and allowed to cool. It deposited a white crystalline substance, m.p. 76° C., which, distilled under diminished pressure (b.p. 198–200° C./3 mm.) and recrystallized from ethyl acetate, formed pearly scales, m.p. 75° C. Calcd. for $C_{26}H_{54}O$: C, 81.67; H, 14.25%. M.W. 382. Found: C, 83.55, 83.67; H, 14.44, 14.43%. M.W. (Rast) 351, 362. This substance therefore consists of ceryl alcohol. Boiled with acetic anhydride in the presence of a trace of pyridine, it forms the characteristic crystalline acetate, m.p. 65° C.

Isolation of Sitosterol

The acetone filtrate from ceryl alcohol was evaporated and the residue dissolved in boiling alcohol. On cooling, the solution deposited a crystalline sterol, m.p. 137° C., which was not further studied.

Isolation of Palmitic and Cerotic Acids

To the alcoholic solution which had been extracted with ligroin, a slight excess of a solution of hydrogen chloride in 85% alcohol was added and the liberated acids extracted by shaking again with several portions of ligroin. The residual alcoholic solution was then diluted with much water and extracted with ether. This extract yielded a light-brown resinous deposit which became crystalline on standing: it amounted to 12% of the wax but could not be further identified. After removal of the solvent from the ligroin solution, the residue (40% of the weight of wax) was boiled with methanolic sulphuric acid and the esters were fractionated under diminished pressure. Two fractions were collected, one boiling at 125° C./3.5 mm., melting at 24–25° C., and identified as methyl palmitate, the other yielding a solid which, after crystallization from alcohol, melted at 68° C. On saponification, this ester produced an acid, m.p. 83–84° C., which, by a mixed melting point determination and analysis, was proved to be identical with cerotic acid. Calcd. for $C_{26}H_{52}O_2$: C, 78.70; H, 13.22%. Found: C, 80.97, 81.06; H, 13.40, 13.44%.

Ether Extract

This extract, amounting to 55.5 gm., was digested with cold ether and the insoluble filtered and washed with ether. The combined filtrate and washings were evaporated to a convenient bulk and extracted successively with 5% aqueous solutions of sodium bicarbonate and of sodium hydroxide. From the residual ether solution nothing definite could be obtained.

The sodium bicarbonate solution on acidification yielded an oil which was converted to the methyl ester; the latter was fractionated under reduced pressure. It yielded a light-yellow oil, b.p. 140–167° C./4 mm. The former, when saponified produced a very small quantity of a crystalline acid, too small to permit further identification.

The sodium hydroxide extract yielded only a small quantity of uncrystallizable gum.

Isolation of Phytosterolin

That portion of the ether extract which was sparingly soluble in cold ether was dissolved in a little dioxane (charcoal) and an equal volume of boiling alcohol added to the concentrated solution. On cooling, a precipitate formed which obviously consisted of a mixture. Several repetitions of this process finally effected the separation of the mixture into a soft, waxy substance and a crystalline compound which could be recrystallized from dioxane-alcohol, m.p. 291–292° C. Calcd. for $C_{33}H_{56}O_6$: C, 72.25; H, 10.21%. Found: C, 72.43; H, 10.25%. This substance therefore is a phytosterol glucoside, and admixture with the similar compound obtained from *A. fungosa* (11) failed to alter the melting point. In Whitby's color reaction it gave a positive test. When boiled with acetic anhydride and a trace of pyridine it forms a crystalline acetate which, recrystallized from methanol, melts at 159° C., and when admixed with the acetate produced from the sterolin of *A. fungosa* (m.p. 157°), melts at 157° C. Furthermore, when hydrolyzed in amyl alcoholic hydrogen chloride, according to the usual method, the sterolin yields a sterol, m.p. 137° C., and glucose, identified as its osazone.

Isolation of a Sterol Ester

The soft, amorphous substance separated from the sterolin was, after repeated recrystallizations from alcohol, still colored brown. It was then refluxed with methanolic sulphuric acid. This treatment removed a small amount of impurity, but did not bring about crystallization of the substance which was eventually boiled with alcoholic potassium hydroxide. The solution was filtered while hot from a little insoluble material and an equal volume of water added. This caused the formation of a slight precipitate which became flocculent as the flask cooled to room temperature. Shaking with several portions of ether removed the precipitate, and the clear solution was acidified and again extracted with ether. The latter extract on evaporation left a small quantity of oil which was esterified, but the yield of product was too small to permit distillation. The ether extract of the neutral substance

after evaporation of the solvent left a crystalline residue which, recrystallized from methanol, melted at 134–135° C. This was boiled with acetic anhydride containing pyridine and the acetate, crystallized from methanol, melted at 130–131° C. Calcd. for $C_{27}H_{45}O.COCH_3$: C, 81.31; H, 11.22%. Found: C, 81.02; H, 11.22%. It gave positive tests in Whitby's color reactions *B* and *C* (15).

Chloroform Extract

After distillation of the solvent, the extract left a resinous mass (36.9 gm.) which was separated by shaking with chloroform into a sparingly soluble part *A* and a part *B*, readily soluble. The chloroform solution of the latter was extracted first with a 5% aqueous solution of sodium bicarbonate and then with a 5% solution of sodium hydroxide. In the course of the extraction with sodium bicarbonate, an insoluble gum *C* separated. This was collected. Nothing but a trace of untractable material could be obtained from the sodium bicarbonate solution.

The sodium hydroxide solution on acidification yielded a bulky precipitate of lignin, which after purification weighed 22.7 gm.

The gum *C* which had separated from the chloroform solution was dissolved in dilute caustic and the filtered solution acidified. Lignin was thus precipitated as a light-brown, granular solid. After repeatedly washing by decantation it was filtered and dried (3 gm.).

The part *A* of the extract which was sparingly soluble in chloroform was boiled with methanol and the mixture filtered while hot. After repeating this twice with fresh portions of methanol, the filtrates were combined and evaporated. The residue was dissolved in dilute caustic and the filtered solution, on acidification, yielded a small quantity (0.9 gm.) of lignin.

From the methanol-insoluble part of Fraction *A*, 8.1 gm. of lignin was isolated in a similar way. Hence 94% of the chloroform extract consisted of lignin.

Ethyl Acetate Extract

According to the difficulty or ease of solubility of the various constituents in ethyl acetate, this extract was separated into two fractions. From an ethyl acetate solution of the readily soluble part of the extract four fractions were obtained, *i.e.*, *A*, soluble in 5% sodium bicarbonate; *B*, soluble in 5% sodium hydroxide; *C*, the neutral residue; and *D*, a gum which separated in the course of the extraction with dilute sodium hydroxide. From Fraction *A*, an oil was precipitated on acidification. It was dissolved in ethyl acetate, dried and esterified with methanolic sulphuric acid. When fractionated under diminished pressure, the ester yielded a light-brown oil, b.p. 155–180° C./4 mm., and a trace of a thick brown oil, b.p. 180–240° C./4 mm. The former was saponified but the product could not be crystallized and was discarded. Nothing tractable could be obtained from Fractions *C* and *D*, but Fraction *B* yielded lignin and so did that part of the original extract which was sparingly soluble in ethyl acetate (4.7 gm.).

Methanol Extract

The solvent was largely distilled and the clear, concentrated extract allowed to stand several weeks at room temperature during which it gradually deposited a crystalline mass which was filtered on a Buchner and washed with cold methanol. The dried deposit weighed 26.8 gm. It was dissolved in a little hot water, and the filtered solution (charcoal) concentrated to incipient crystallization and allowed to cool. Recrystallized from alcohol, it formed feathery needles, m.p. 340° C., identified as potassium nitrate.

Isolation of Lignin

The original methanolic filtrate from which the potassium nitrate had been filtered was poured into four litres of hot water and the flocculent precipitate filtered with suction and washed with water. The light-brown, amorphous powder was dissolved in dilute caustic, the solution filtered and acidified to Congo red with dilute hydrochloric acid. A granular precipitate formed immediately and was washed by decantation with hot (80° C.) water. Filtered and dried, it amounted to 7.2 gm. and was found to consist of lignin.

Isolation of i-Inositol

A warm solution of basic lead acetate was poured into the dark-brown aqueous-methanol filtrate from which the lignin had been removed and the precipitate filtered off. After de-leading with hydrogen sulphide, and removal of the lead sulphide, the filtrate was concentrated under reduced pressure to a syrup which was boiled with methanol and again concentrated. A large proportion was then insoluble in absolute methanol. The soluble portion yielded an additional 4 gm. of potassium nitrate. A sample of the syrup failed to yield a crystalline osazone when treated with phenylhydrazine.

The brownish-yellow lead precipitate was suspended in water and de-leaded with hydrogen sulphide, the lead sulphide filtered off and the filtrate concentrated to a syrup under reduced pressure. This was dissolved in methanol and again evaporated to a syrup, the process being repeated several times. Finally, the syrup was dissolved in methanol (charcoal) and the solution allowed to stand at room temperature. It deposited small, stout, colorless crystals which produced the usual salmon color in Sherer's test for inositol. Recrystallized from boiling alcohol it melted at 224–225° C. Calcd. for $C_6H_6(OH)_6$: C, 40.00; H, 6.67%. Found: C, 39.70; H, 6.63%. The presence of *i*-inositol in wheat straw, therefore, is established.

Aqueous Extract

All efforts to isolate the coloring matter present in this liquor failed. Extraction with various solvents did not remove anything and even treatment with basic lead acetate yielded nothing but trifling amounts of intractable gums.

Dilute Caustic Soda Extract

After filtration the dark brown solution was acidified with dilute hydrochloric acid and the flocculent precipitate washed repeatedly with hot water

(80° C.) by decantation and on the filter. This precipitate, which consisted of lignin, yielded a brown amorphous powder (15 gm.) which could be methylated with dimethyl sulphate and 50% potassium hydroxide, yielding a methyl derivative no longer soluble in sodium hydroxide.

Acknowledgment

The author wishes to acknowledge his indebtedness to Mr. C. W. Davis, of these laboratories, who determined all the iodine numbers.

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MEASUREMENT OF THE HEATS OF WETTING OF CELLULOSE AND WOOD PULP[1]

By G. H. Argue[2] and O. Maass[3]

Abstract

The heats of wetting of dry cellulose and wood pulp have been measured for the first time. The influence of the initial water content of the cellulose on the heat of wetting has been determined. It is shown that when the water is left as a result of partial desorption a higher value is obtained. The heats of wetting of wood meal and different wood pulps have been determined. The heats of adsorption were calculated, and all the data are discussed from the point of view of the physical structure of cellulose. A few preliminary measurements of the heat of adsorption of sodium hydroxide were made. The experimental technique of using a rotating adiabatic calorimeter is shown to be admirably adapted for measurements of this kind.

Introduction

Information regarding the colloidal system cellulose-water is not only of scientific interest but of practical importance. Adsorption and desorption isothermals have been measured (13-15), and volume changes which take place on the addition of water to cellulose have been observed (3). From the data thus obtained inferences have been drawn as to the mechanism of water adsorption and the colloidal structure of cellulose. The purpose of the present investigation was to gain more information regarding the nature of water adsorption on cellulose by measuring the heat of wetting, and to correlate this with the conclusions drawn from the first-mentioned data. In this connection it was realized that it would be important to carry out measurements of the heat of wetting of cellulose pretreated in different ways.

There are few data available. The heats of wetting of several cellulose acetates have been indirectly determined by Newsome and Sheppard (9). Katz (5, 6) made direct measurements on the heat of wetting of one sample of cellulose. The accuracy of the technique developed in this laboratory (7) for the measurement of small heat changes opened the way for a thorough investigation of the heat of wetting of cellulose.

Apparatus

The apparatus employed in this work was essentially the same as that first used by Lipsett, Johnson and Maass in the determination of the heat of solution of sodium chloride in water, and in the measurement of the surface energy of solid sodium chloride (7, 8). It consists of a small rotating adiabatic calorimeter with a small inner silver box, the latter having a removable cover. The purified cellulose was placed in this, a definite weight of water was put in the silver calorimeter and the apparatus was assembled. Temperature control between the inner and outer calorimeter was maintained by the use of

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a multiple-junction, radiation thermocouple of copper-constantin. The temperature of the water bath was measured by means of a platinum thermometer with a Mueller bridge and a sensitive galvanometer. For details of the apparatus, its manipulation and calculation of results with the necessary corrections, the reader is referred to the original papers (7, 8).

Considerable trouble in removing the cover of the box containing the cellulose was experienced in the early experiments. The amount of vaseline that it was necessary to use in order to prevent water vapor from gaining access to the cellulose prevented the cover from falling off when rotation began. To obviate this the cover of the inner silver box was made of iron. This was silver plated to prevent rusting. A strong electromagnet of the horseshoe type was mounted in the outer water bath directly over the lid of the calorimeter. When temperature equilibrium had been attained and the initial temperature measured, a current was passed through the electromagnet and the rotation started simultaneously. The pull on the lid due to the magnetic field, added to that due to the rotation, dislodged the lid in spite of a thick layer of vaseline. Thereupon the magnet was immediately removed from the water bath, so that the heat generated by the electric current would not interfere with the adiabatic control.

Purification of Cellulose

The cellulose was prepared from pure cotton. This was placed in a flask and refluxed with a 1% sodium hydroxide solution for 12 hr. The liquor was poured off, the sample washed with distilled water and the procedure repeated seven times. The cellulose was then acidified with a 1% acetic acid solution and washed free of acid with distilled water. The excess water was pressed from the cotton and the purified sample was air dried for a day, then placed in an oven at 100° C. for two days and finally was transferred to a storage bottle. In this paper, cellulose prepared in this way will be designated as standard cellulose.

Procedure

An adequate amount of cellulose was used and the fibres were separated as much as possible. This was heated for 3¼ hr. in an electric oven maintained at $100 \pm 0.5°$ C. The sample was then transferred to the silver box while in the oven, and the lid was placed in position. Experiments showed that no moisture from the air was taken up by the cellulose during this operation. The weight of the sample after this treatment was considered as the dry weight of the cellulose, and all calculations are based on this degree of dryness. Approximately 50 cc. of water was placed in the calorimeter from a weight pipette, and the apparatus was assembled.

After thermal equilibrium had been attained the temperature was read for 10 to 15 min. and could be maintained constant to $\pm 0.0001°$ C. When the reaction began, the evolution of heat in the calorimeter was compensated for by the addition of hot water to the outer bath. The rotation was continued for 20 min., temperature equilibrium being maintained during this time.

The rotation of the calorimeter was stopped and the final temperature was measured for a period of time. In order to correct for the mechanical heat generated during the rotation, the calorimeter was rotated a second time and the temperature rise per minute was calculated and this correction applied to the resultant temperature.

In some experiments the initial water content of the sample was brought to a definite value. Here the dry weight of the cellulose was found by first weighing the sample in a small glass weighing bottle under conditions as described above. The cellulose was transferred to the silver box and allowed to absorb moisture from an atmosphere containing a definite amount of water vapor. The sample was then weighed in the closed silver box. The weights of cellulose and water were converted to those *in vacuo*, and the weight of water per gram of dry cellulose was calculated.

In the experiments which are labelled "Desorption", the dry weight of cellulose was determined as described above. The sample was saturated with water, placed in the open silver box and heated in a vacuum desiccator over phosphorus pentoxide to 50° C. The time for this treatment was adjusted so as to leave a desired amount of moisture on the sample. The lid was replaced and the exact amount of water determined by weight.

The heat constant for the silver calorimeter was determined by measuring the heat of solution of sodium chloride in water and making use of the data obtained by Lipsett, Johnson and Maass (7, 8). The specific heats of the component parts being known, a calculation of the heat capacity of the calorimeter was made in this way and found to agree with that obtained by direct measurement. The specific heat of cellulose was considered to be 0.32 cal. per gram (4).

The temperature change of the calorimeter could be measured to within 0.0002° C., and as the temperature change varied from 0.2 to 0.08° C., depending on the initial amount of water adsorbed, an accuracy of from 0.1 to 0.5% was possible in the heat measurements. The average weight of the cellulose sample taken was 2 gm., and since weighing could be carried out to 0.1 mg. the weight of water adsorbed could be estimated to 0.5% for a sample having an initial water content of 1%, and more accurately for the initial water adsorptions.

Results

The initial values found for the heat of wetting varied considerably more than could be accounted for by the calculated limit of error. These early experiments were carried out with cellulose samples that had not been treated in a uniform manner, both with regard to purification and uniformity of water adsorption. The magnitude of the heat measured depends on this and on other influences to which the cellulose had been subjected previous to the measurement. As soon as this was realized the exact procedure of pretreatment as outlined above was followed and duplicate checks could be obtained. The data shown in Table I for standard cellulose are the averages of approxi-

mately 50 determinations made with samples which had been subjected to identical chemical and heat treatment before the measurement was made. These results are shown graphically in Fig. 1.

The range of concentration of adsorbed water covered for the heat of wetting was 0.0 to 0.07 gm. of water per gram of cellulose. Of the various determinations, those with dry cellulose could be made most readily and afforded the best checks. With concentrations of adsorbed water greater than 0.07 gm. it was difficult to bring about uniform adsorption on the cellulose, and, as the results beyond this concentration are of lesser interest, no attempt was made to modify the procedure in order to make these measurements possible.

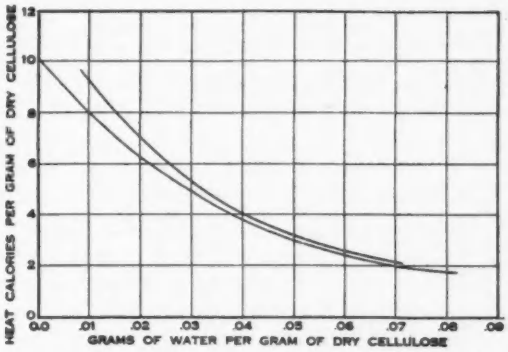

FIG. 1. *Heat of wetting of cellulose containing adsorbed water and that of cellulose containing water left as a result of partial desorption.*

TABLE I

EXPERIMENTAL RESULTS ON THE HEAT OF WETTING OF CELLULOSE CONTAINING ADSORBED WATER AND THAT OF CELLULOSE CONTAINING WATER LEFT AS A RESULT OF PARTIAL DESORPTION

Adsorption		Desorption		Adsorption		Desorption	
Water content, gm.	Heat of wetting, cal.	Water content, gm.	Heat of wetting, cal.	Water content, gm.	Heat of wetting, cal.	Water content, gm.	Heat of wetting, cal.
0.0	10.16			0.04	3.78	0.04	4.04
0.005	9.0			0.05	2.98	0.05	3.20
0.01	8.0	0.01	9.29	0.06	2.39	0.06	2.64
0.02	6.27	0.02	7.1	0.07	1.99	0.07	2.17
0.03	4.9	0.03	5.32	0.08	1.78		

NOTE:—*Water content is the weight of water per gram of dry cellulose present before measurement of the heats of adsorption and desorption. Heat of wetting is the heat of wetting per gram of dry cellulose in the sample.*

The desorption range was from 0.015 to 0.07 gm. of water per gram of dry cellulose. At lower concentrations the results were not consistent and cannot be expected to be so, as will be explained later.

From the data in Table I and its graphic presentation a number of calculations can be made. Since the heat of wetting of dry cellulose is 10.16 cal. per gram of cellulose, the heat of adsorption H_1 given out when x grams of water is added to 1 gm. of cellulose is obtained by subtracting the heat

of wetting of 1 gm. of cellulose containing x grams of water from 10.16 cal. These data are given in Columns 1 and 2 of Table II.

These results can be put in another way: $1/x$ is the number of grams of cellulose on which 1 gm. of water will give an amount of adsorbed water equal to the concentration given in Column 1. H_1/x will then represent the amount of heat given out when 1 gm. of water is added to $1/x$ grams of cellulose. The fifth column of Table II shows the heat of adsorption of 1 gm. of water vapor on cellulose containing per gram the amounts of water shown in Column 1, the weight of cellulose being such that the addition of this water does not change the amount of adsorbed water. This heat may be calculated as follows:— if dx gm. of water, added to 1 gm. of cellulose containing x grams of water, gives out dH_1 calories, 1 gm. of water on $1/dx$ gm. of cellulose will give out dH_1/dx calories on cellulose containing x grams of water per gram of cellulose. dH_1/dx is the heat of adsorption of 1 gm. of liquid water on an infinite amount of cellulose at this water concentration. The values of dH_1/dx can be obtained by differentiating the equation

TABLE II

CALCULATIONS OF THE HEAT OF ADSORPTION OF WATER VAPOR ON CELLULOSE

x, gm.	H_1, cal.	$1/x$, gm.	H_1/x, cal.	H_2, cal.
0.0	0			
0.005	1.16	200	232	
0.01	2.16	100	216	775
0.02	3.89	50	194.5	740
0.03	5.26	33.4	175.3	697.9
0.04	6.38	25	159.5	668.9
0.05	7.18	20	143.6	651.6
0.06	7.77	16.7	129.5	639.9
0.07	8.17	14.3	116.7	631.7
0.08	8.38	12.5	104.8	625.5

NOTE:—*x is the weight of water adsorbed per gram of dry cellulose, H_1 the heat of adsorption of x gm. of water on 1 gm. of cellulose, $1/x$ the weight of dry cellulose required per gram of water to give adsorption x, H_1/x the heat of adsorption liberated when 1 gm. of water is added to $1/x$ gm. of cellulose, and H_2 the heat of adsorption of 1 gm. of water vapor on an infinite amount of cellulose containing x gm. of adsorbed water.*

$$H_1 = 8.01 \log x + 17.49,$$

which accurately represents the data for the range 0.02–0.07 gm. of water per gram of cellulose. For values less than 0.02 gm. recourse was had to graphic estimation of the tangent to a curve representing the data of Columns 1 and 2. To dH_1/dx is added 582 cal., the heat of condensation of water at 25° C.

In Fig. 2 is shown the heat of adsorption of water vapor on cellulose containing various amounts of adsorbed water. The data in Column 4 of Table II plus 582 cal. when extrapolated to zero concentration should give the same values as those of the heat of adsorption of water vapor on dry cellulose. The upper curve representing these data shows that this is the case, a value 250 cal. greater than the heat of condensation of water vapor being obtained for the heat of adsorption of 1 gm. of water under these conditions. It is seen from the figure that the heat of adsorption falls rapidly with increasing concentration of adsorbed water, and will eventually become the same as

the value of the heat of condensation of water vapor. The greatest change takes place during the adsorption of the first 0.04 gm. of water per gram of cellulose.

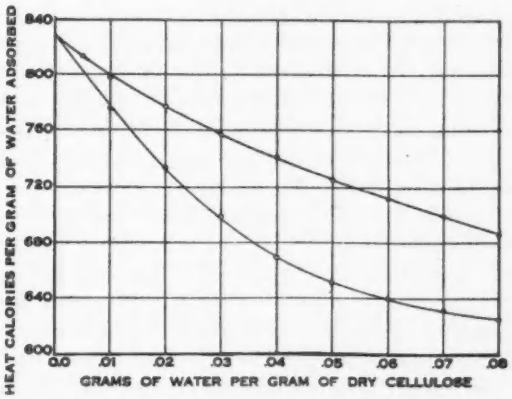

FIG. 2. *Heat of adsorption in calories of 1 gm. of water vapor on cellulose containing various amounts adsorbed water.*

Discussion

For clarity in discussion of the experimental results it is necessary to present the authors' views regarding the physical structure of cellulose. Cellulose consists of long chains made up of a hundred or more glucose units. A number of these chains (approximately 40 or more) are symmetrically connected together in a bundle to form sub-microscopic crystals or micelles. The fibre of cellulose is composed of these micelles which are in contact with one another over a considerable part of their surface. Where contact occurs, crystal forces similar to those holding the chains in the micelle come into play. The remaining micelle surface may conveniently be called the inner free surface of the cellulose.

Gas molecules can penetrate into the fibre, reaching this free surface, the cellulose crystals themselves remaining impenetrable. Adsorption of gases can take place on this free surface, and the first portion of water vapor to enter a dried fibre is adsorbed in this manner. The water vapor entering subsequent to the saturation of this surface will condense and fill the intermicellar spaces. The amount of water held by the cellulose for a given vapor pressure depends on whether water has been added to the dried cellulose or abstracted from cellulose saturated with water vapor. In other words, adsorption is followed by hysteresis when desorption takes place.

Because of this and the form of the adsorption isotherm it follows that the water also increases the inner surface in the sense that contact areas between micelles are disrupted, forces between cellulose and water replacing those of cellulose to cellulose. Since cellulose does not disperse into its micelles when placed in liquid water, the cellulose-to-cellulose attraction must be greater than the cellulose-to-water. Hence the cellulose-to-cellulose bonds that are disrupted by the water are those which are under a particular strain due to the manner in which the micelles are arranged in the fibre. Thus, after the water vapor required for saturation of the originally present inner free surface has been adsorbed, subsequent addition of water vapor creates more surface and the lowering of the vapor pressure is greater than need be accounted for by the curvature of the liquid water in the intermicellar spaces. The form of the sorption curves is in agreement with this. Furthermore, on desorp-

tion the first water removed is that in the intermicellar spaces. Water held on the freshly created surface will require a lower vapor pressure to be removed than would be the case if the water were in the intermicellar spaces. When this water is removed the cellulose surfaces can join up again.

The foregoing hypothesis of the mechanism of water adsorption is in many respects similar to that proposed by Urquhart (12). The idea of strains helping to disrupt micelle surfaces from one another was first put forward by Campbell to correlate the adsorption isotherms of water, and propyl and methyl alcohols (1, 2).

In addition to the above it is reasonable to suppose that the inner free surface is not uniform in regard to its absorbing power. For instance, the ends of the molecular chains will be fundamentally different from the sides, and presumably present a greater amount of free valency for adsorption purposes. Over all the surface there will be active groups affording regions on which there will be pronounced adsorption. From such centres the tendency to adsorption gradually falls off.

When liquid water is brought into contact with cellulose, water is adsorbed, giving rise to an evolution of heat; at the same time the surface is increased. This requires energy for the splitting of the cellulose-to-cellulose bonds between micelles. The greater the amount of water initially present in the cellulose the smaller are both effects. This explains the data obtained from the "desorption" and "adsorption" experiments and shown graphically in Fig. 1. Cellulose from which a portion of the water has been desorbed has a larger surface than cellulose containing the same amount of water added to a dry sample. When liquid water is brought into contact with these two samples both will have the same final surface area. Hence in the former case less energy is required for surface formation, and consequently the heat of wetting is greater. When water is desorbed from wet cellulose to such an extent that there is less than 0.02 gm. of water per gram of dry sample, cellulose reverts to the dry state, and the extent to which it does is dependent on the rate of water removal and experimental conditions. Thus, reproducible experimental values could not be obtained in this region, as was pointed out in the experimental section. When 1 gm. of water vapor is adsorbed on an infinite surface of dry cellulose, 250 cal. more than the heat of condensation is given out. On a sample prepared differently this limiting value was found to be 280 cal. It would appear therefore that these two cellulose surfaces differ from each other, and this is in agreement with the hypothesis stated above. If the adsorption were not uniform in intensity on the different parts of the molecular chains, samples having different chain lengths and different micelle distributions would give rise to differences in the average intensity with which sorption takes place on their surfaces.

The heat of adsorption per gram of water falls rapidly with an increase in the moisture content of the cellulose, reaching one-fifth of the value for dry cellulose when the cellulose contains one-third of the quantity of water required for saturation of the fibre—in this case 0.07 gm. water per gram of

cellulose. At this stage the relative vapor pressure is 50%, a reduction in vapor pressure which would be given by capillaries having a diameter corresponding to approximately ten water molecules. The general shape of the curve for heat of adsorption will be discussed in another paper, in correlation with dielectric constant determinations on cellulose containing various amounts of adsorbed water.

When the heat of adsorption of 1 gm. of water per infinite amount of cellulose is calculated from the desorption experiments, it is found that the the heat of adsorption is definitely greater when adsorption takes place on a cellulose sample from which a portion of the water has been desorbed. Thus when 0.02 gm. of water per gram of cellulose has been left after desorption it is 773.5 cal. as compared to 733 cal. When the water content is 0.03 gm. of water per gram of cellulose the values are 709.7 and 687.9 cal. This shows that a larger surface is available on cellulose from which water has been desorbed.

In a recent paper Stamm (10) has calculated the heats of adsorption from vapor pressure isothermals. It is of interest to do the reverse and calculate the isothermals from the heats of adsorption. Data of Urquhart and Williams (13-15) are taken for this purpose. The 25 and 100° C. isothermals are taken and the latter calculated by means of the equation

$$H = \frac{1.98 \times 298 \times 373 \times 2.3}{18\,(373-298)} (\log h_2 - \log h_1),$$

where H is the heat of adsorption given by the measurements of the heat of wetting in Table II, and h_1 and h_2 are the relative humidities.

In Table III the first column shows the moisture content, the second and third columns the experimental data of Urquhart and Williams and the fourth column the humidity at 100° C., calculated from our data.

TABLE III

CALCULATION OF THE ISOTHERMAL VAPOR PRESSURE FROM HEAT-OF-WETTING DATA

Moisture content, gm. water/gm. dry cellulose	h_1 (25° C.)	h_2 (100° C.)	h_2 (100° C.) Calcd.
0.02	0.10	0.242	0.253
0.03	0.222	0.390	0.449
0.04	0.340	0.550	0.580
0.05	0.45	0.656	0.689
0.06	0.57	0.745	0.814

It is seen that the agreement over the range 0.02–0.05 gm. of water per gram of cellulose is good. Below this range the calculated values are too high and above this range they are too small. The assumption made in the calculation, that the heat of adsorption is independent of the temperature, cannot be strictly true. It is also apparent that the heat of wetting of cellulose containing less than 0.02 gm. of water cannot be calculated with any accuracy from the isothermals. The variation of the heat of wetting with the water content is greatest here, and the data for the amount of adsorption are the least accurate in this region. That the heat of wetting of dry cellulose is of particular interest will be made apparent in the following section.

Heat of Wetting and the Physical Structure of Cellulose

The heat of wetting of wood pulp is considerably larger than that of cotton cellulose. Nine samples obtained from different cooking processes were examined and the heat of wetting was found to lie between 12.3 and 13.3 cal. The specific viscosities of 1% solutions of these pulps in cuprammonium were measured, as also was the viscosity of a sample of cotton pulp whose heat of wetting was found to be 10.5 cal. Assuming Staudinger's theory, that these viscosities show the length of molecular cellulose chains, to be correct, no relation was found between the length of chain as given by this viscosity and the heat of wetting.

The viscosities of xanthate solutions of these pulps, prepared under identical manufacturing conditions, were measured by the falling sphere method. The pulp having the largest heat of wetting had the smallest viscosity in xanthate solution, 5.1 poises, whereas the cotton pulp had the largest viscosity, 10.7 poises. Within the accuracy of measurement, the viscosities in xanthate solution and the heats of wetting of the other samples followed the same sequence.

When the cellulose is converted into viscose, the chains are broken down during the shredding and aging periods, and at the same time secondary forces between the micelles are broken up owing to the action of caustic soda, atmospheric oxygen and carbon disulphide. The viscosity of viscose indicates the extent to which the chains can be separated from one another. A relatively low viscosity means a breaking up into smaller particles. This is more likely to happen the greater the internal surface. The greater heat of wetting corroborates this. The extent of the internal surface is therefore dependent more on the number of chains to the micelle than on the length of the chains. This is to be expected, for the length of a micelle is many times as great its diameter. The heat of wetting may prove to be of interest in predicting the extent of viscose dispersion, and experiments in this direction are contemplated.

TABLE IV

HEAT OF WETTING PER GRAM OF DRY CELLULOSIC MATERIALS (CAL.)

Standard cellulose	10.16
Cotton pulp	10.54
Kraft pulp	13.08
Bleached sulphite pulp:—	
Beating time, min. 0	12.68
Beating time, min. 10	13.01
Beating time, min. 20	13.48
Beating time, min. 30	13.61
Beating time, min. 40	13.92
Wood meal:—	
Sapwood, −100 mesh	19.78
−40 +100 mesh	19.26
Heartwood, −100 mesh	18.41
−40 +100 mesh	18.13

In Table IV the heats of wetting of a number of cellulosic materials are shown in the second column. In the first column the nature and, if necessary, the special condition of the material are indicated.

It is of interest to note that apparently the heat of wetting of a pulp increases only slightly with beating. In the beater, the outside area of the pulp particles is increased 100-fold, but as the internal area of the smallest particle formed is probably thousands of times its external area, the total area available for

wetting is increased only to an insignificant extent by the increased subdivision. What increase in the heat of wetting has been found must therefore be due to an increase in the internal area during beating. Such an increase is to be expected, as the mechanical stresses to which the fibres are subjected during the beating may well bring about a loosening of some micellar bonds.

Campbell (1, 2) has shown that the amount of adsorption of water does not increase during beating to the extent that would be expected on the basis of the "hydration theory of beating" (11), and he showed that this theory was untenable. His recent accurate determinations show an average increase of about 4% in adsorption for pulp beaten to the same extent as the sample mentioned in Table IV. The heats of wetting given above show a somewhat greater percentage increase. In a number of other samples of beaten pulp the increase in the heat of wetting was found to vary from 2 to 10% for 40 min. of beating, but since the beating conditions were not uniform these are not tabulated and mention of them is made simply to show the order of magnitude of the increase in the heat of wetting.

The heat of wetting of wood meal is much greater than that of cellulose, as the figures show; that of sapwood is slightly greater than that of heartwood, and increased subdivision apparently causes a small increase in the heat of wetting.

The adsorption of water vapor on standard cellulose, bleached sulphite pulp and wood meal has been found to be 0.060, 0.070 and 0.10 gm. of water vapor per gram of dry material respectively at 50% relative humidity. The proportionality factor to the data on the heat of wetting is 1.70, 1.8 and 1.84 respectively. As might be expected the adsorption is practically proportional to the heat of wetting. This is in agreement with the fact that the heat of wetting of cellulose from which a portion of the water has been desorbed is greater than that of cellulose containing an equal amount of water due to adsorption as shown previously.

The apparatus described is admirably adapted for other investigations of a nature similar to the foregoing. Some measurements have also been made on the heat of wetting of cellulose in sodium hydroxide solutions. Standard cellulose containing approximately 5% of water showed a heat of wetting of 12.6 cal. per gram of dry cellulose in a 2% solution of sodium hydroxide. In more concentrated solutions the heat of wetting was greater, being 21.5 and 13.3 cal. for dry samples in 16 and 8% solutions of sodium hydroxide. The measurement of the heat of wetting of cellulose in alcohols is under way, and in a number of preliminary experiments it has been found that the heat of wetting with 95% methyl alcohol is 6.2 cal. per gram of cellulose.

It will be interesting to make measurements of heats of wetting with alcohol solutions containing varying amounts of water. It is hoped that these will throw further light on the validity of the interpretation of the experimental results which have tentatively been made by the authors.

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